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Synthesis and Anti-inflammatory Activity of 2,6-Di-*tert*-butylphenols with a Heterocyclic Group at the 4-Position. III.[1)]

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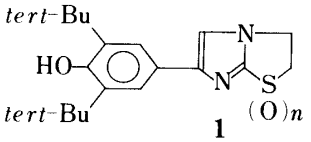
A series of 2,6-di-*tert*-butylphenols with azoles at the 4-position was synthesized and evaluated for anti-arthritic activity in adjuvant-induced arthritis (AA) assay. Some compounds were also examined for anti-inflammatory activity in carrageenin-induced rat paw edema assay (CIPE) and for analgesic activity in AcOH-induced writhing assay in mice. 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-methyl-2-oxo-4-imidazoline (**6b**) (25 mg/kg, *p.o.*) had about the same activity as indomethacin (2 mg/kg, *p.o.*) in AA assay. Compound **6b** (25 mg/kg, *p.o.*) was as potent as phenylbutazone (50 mg/kg, *p.o.*) and indomethacin (3 mg/kg, *p.o.*) in CIPE and showed low acute toxicity (> 1000 mg/kg, mouse, > 400 mg/kg, rat). Compound **6b** had radical-scavenging activity *in vivo* and *in vitro*, and showed mild inhibitory activity on delayed-type hypersensitivity. Thus **6b** is a promising candidate as a new anti-arthritic agent. Detailed pharmacologic studies of **6b** are under way.

Keywords——anti-inflammatory activity; 2,6-di-*tert*-butylphenol; thiazole; imidazole; imidazoline; azole; analgesic activity

A number of nonsteroidal anti-inflammatory drugs (NSAID) have been used in the treatment of rheumatoid arthritis, but their clinical efficacy is generally insufficient. Recent studies[2)] of inflammation suggest that oxygen-derived radicals play an important role at the inflammatory site. Moreover superoxide dismutase,[3)] which has radical-scavenging activity, showed clinical efficacy in treating patients with rheumatoid arthritis. These facts indicated that compounds which have radical-scavenging activity may be a new type of anti-inflammatory agent. Therefore we have studied the anti-inflammatory activity of 2,6-di-*tert*-butylphenols with a heterocyclic group at the 4-position and reported in the previous paper[1)] that 6-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2,3-dihydroimidazo[2,1-*b*]thiazoles (**1**), especially the sulfoxide **1b**, had potent anti-inflammatory and analgesic (AcOH-induced writhing assay) activities and that anti-inflammatory activity of these phenols largely depended on the heterocyclic group at the 4-position. There have been a number of reports on the anti-inflammatory activity of azole compounds[4-8)] and the heterocyclic moiety of **1a** could be regarded as an imidazole derivative because ring fission of the heterocyclic moiety of **1a** would yield 2-alkylthioimidazole. Therefore in this paper we describe the synthesis and anti-inflammatory activity of 2,6-di-*tert*-butylphenols with an azole moiety at the 4-position.



1

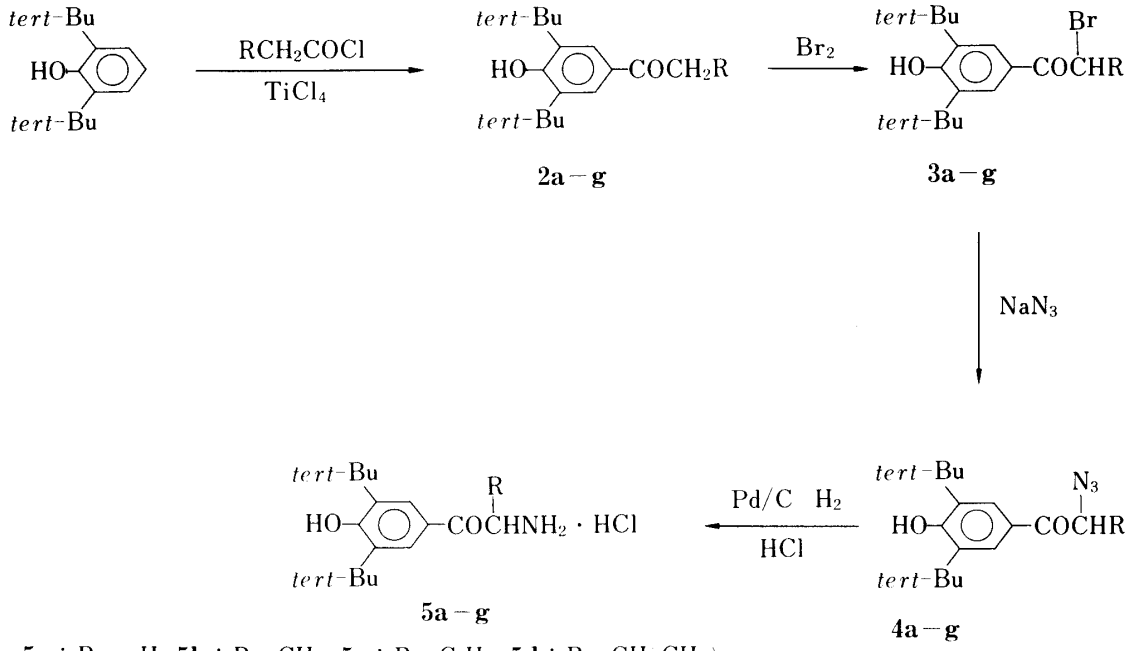
1a : $n=0$
1b : $n=1$
1c : $n=2$

tert-Bu = $C(CH_3)_3$

Fig. 1

Chemistry

4-(2-Aminoacyl)-2,6-di-*tert*-butylphenols (**5a**—**g**) were easily prepared by reacting the corresponding 4-(2-bromoacyl)-2,6-di-*tert*-butylphenols (**3a**—**g**)[9] with sodium azide in methanol, followed by catalytic hydrogenation using palladium charcoal as a catalyst.



5a : R =H, **5b** : R=CH_3, **5c** : R=C_2H_5, **5d** : R=$CH(CH_3)_2$
5e : R =$CH_2CH(CH_3)_2$, **5f** : R=C_6H_{11}, **5g** : R=C_6H_5
tert-Bu=$C(CH_3)_3$

Chart 1

Treatment of compound **5** with potassium isocyanate or sodium thiocyanate gave 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-oxo-4-imidazolines (**6a**—**g**) and 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-thioxo-4-imidazolines (**6h**—**l**), respectively. Desulfurization of compounds **6i**—**l** with Raney nickel afforded 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)imidazoles (**7a**—**d**). Compounds (**6h**—**l**) were allowed to react with appropriate alkyl halides to yield the corresponding 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-alkylthioimidazoles (**8a**—**j**). Tetrafluoroalkylthio derivatives (**8k** and **8l**) were prepared in poor yields by reacting compound **6i** or **6j**, respectively, with tetrafluoroethylene by a method similar to that described by Rapp *et al.*[10] Oxidation of compounds **8a**, **8b** and **8k** with *m*-chloroperbenzoic acid gave the corresponding 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-methylsulfinylimidazole (**9a**) and 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-alkylsulfonylimidazoles (**9b**, **9c**, and **9d**). 5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-mercapto-1-methylimidazole (**10a**) was prepared by reaction compound **5a** with methylthiocyanate under heating in pyridine, and 5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-hydroxy-1,4-di-methylimidazole (**10b**) was prepared from compound **5b** with methylisocyanate in a similar manner.

Compounds thus prepared were evaluated for anti-inflammatory activity in adjuvant-induced arthritis assay. Since some compounds showed potent anti-inflammatory activity, various thiazole compounds were prepared because compound **1a** has a thiazole ring in its structure. 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-mercaptothiazoles (**11a**—**c**) were prepared from compound **3** with ammonium dithiocarbamate using a modification of the procedure described by Ritter *et al.*[11] Compounds (**11a**—**c**) were also converted to the corresponding 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-alkylthiothiazoles (**12a**—**j**) by reaction with the appro-

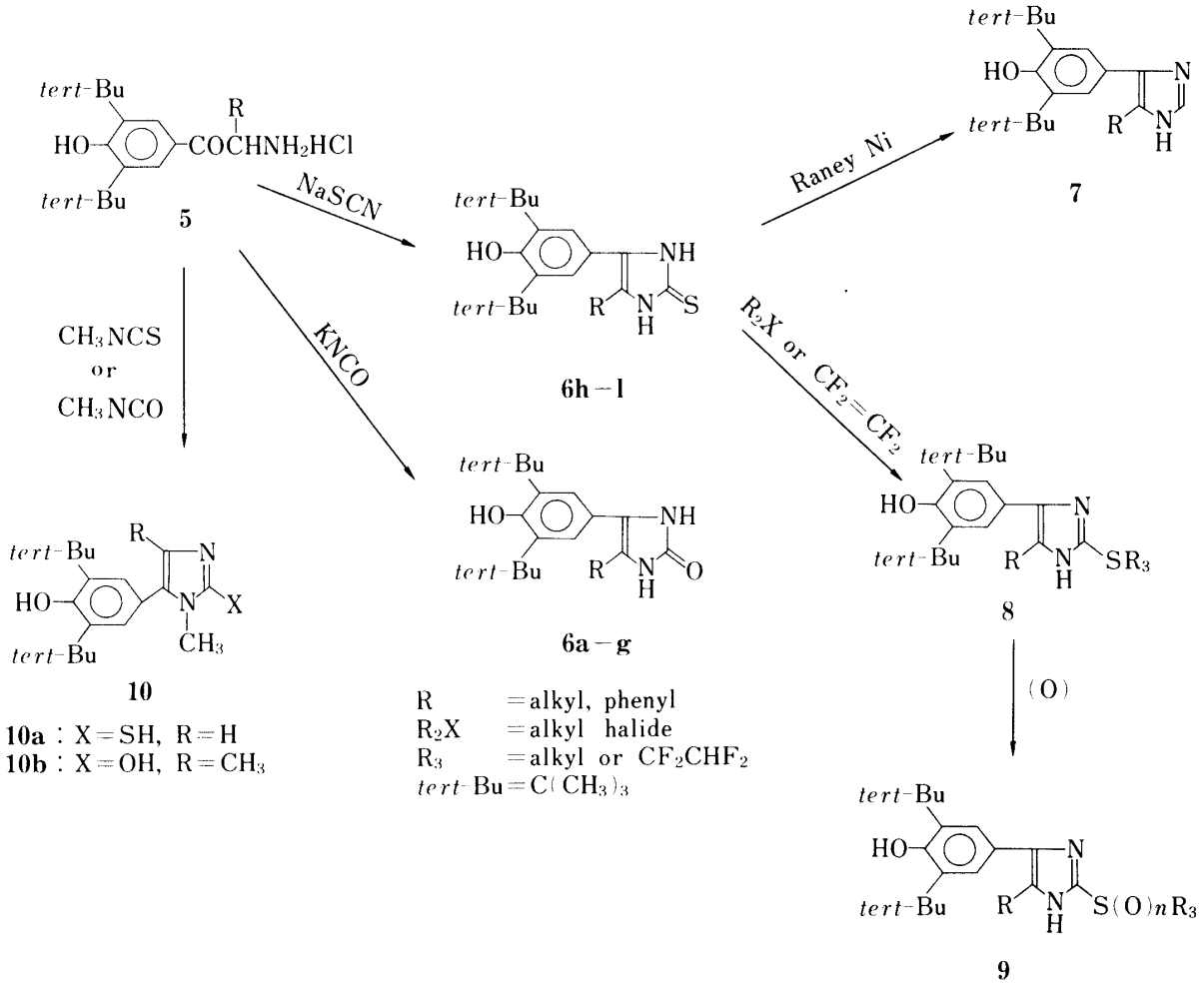


Chart 2

priate alkyl halide or tetrafluoroethylene. Compound **12a** was oxidized with *m*-chloroperbenzoic acid to yield 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-methylsulfinylthiazole (**13a**) and 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-methylsulfonylthiazole (**13b**). Compounds **14**—**18** were prepared in order to compare the potency of anti-inflammatory activity with that of compound **7a**. 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-methylthiazole (**14a**) and 4-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-5-methyloxazole (**14b**) were prepared by reacting compound **3b** with thioformamide or formamide, respectively.

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methylpyrrole (**15**) was prepared by reacting compound **5b** with acetylene dicarboxylic acid dimethyl ester, followed by decarboxylation with lithium iodide in collidine. 5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-4-methyl-3-methylthiopyrazole (**16**) was prepared from compound **3b** and *S*-methyldithiocarbazide according to the method described by Beyer *et al.*[12] Compound **16** was also converted to the corresponding sulfoxide **17** by oxidation. 5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-4-methylisoxazole (**18**) was prepared in poor yield from compound **2b**, *N*,*N*-dimethylformamide diethylacetal and hydroxylamine-*O*-sulfonic acid according to the method described by Lin-i *et al.*[13]

Compounds **6a**—**l**, **7a**—**14b** and **15**—**18** are listed in Tables I—III, respectively.

Pharmacology and Discussion

Biological activity of all compounds (*p.o.*, 25 mg/kg) was evaluated by using adjuvant-induced arthritis (AA) assay and compared with that of indomethacin (*p.o.*, 2 mg/kg). Among

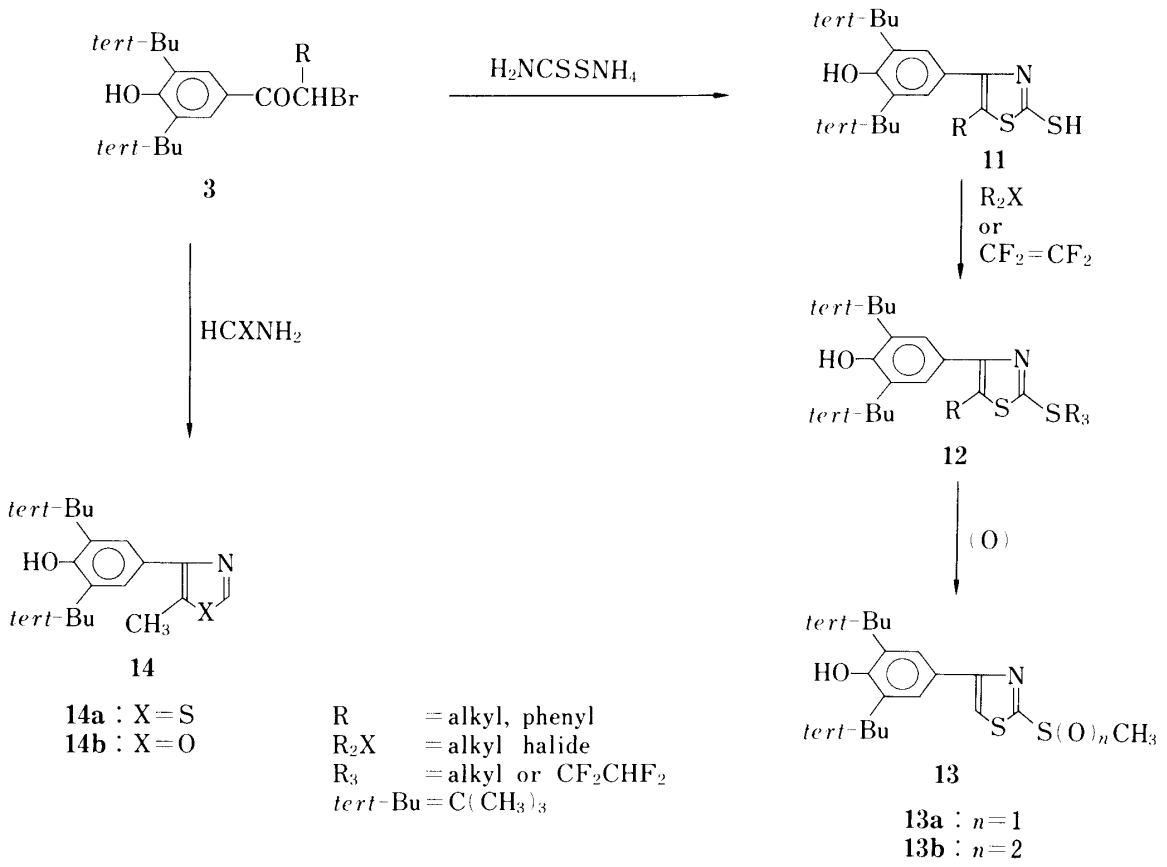


Chart 3

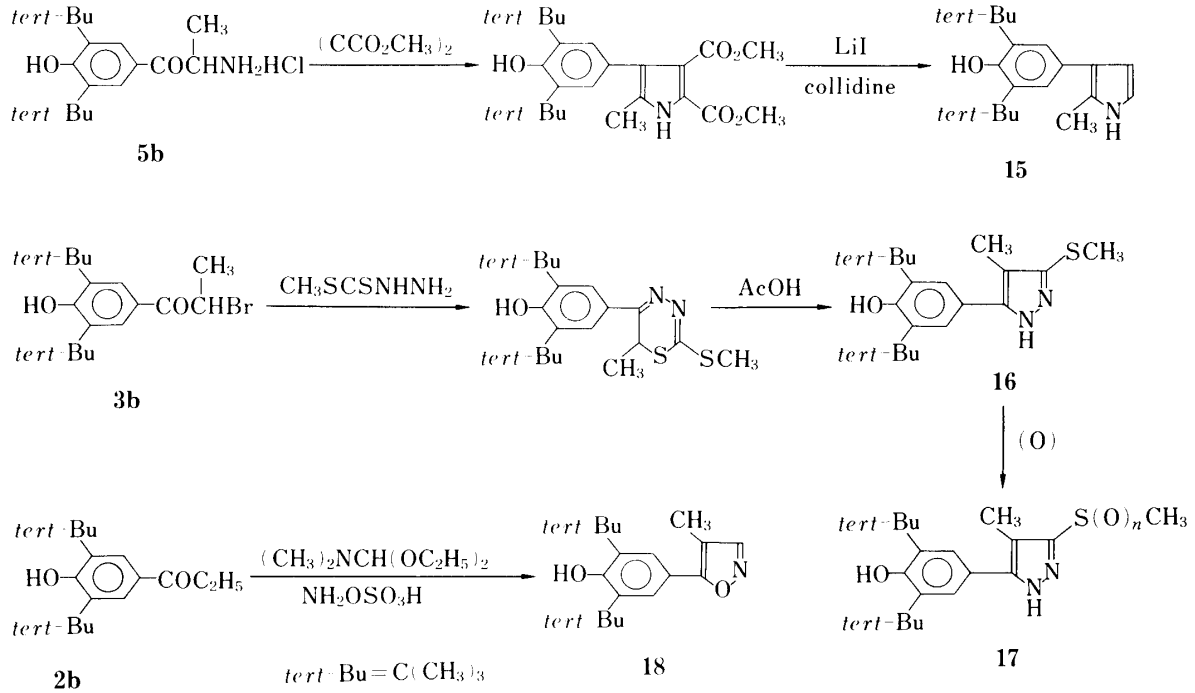
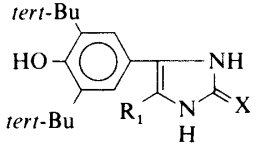


Chart 4

the compounds, **6b**, **6c** and **12a** showed strong activity. Among the compounds which have a substituent at the 5-position of **6a** (compounds **6b**—**g**), **6b** and **6c** showed potent activity, but **6d**—**g** which have a bulky substituent did not show any activity at 25 mg/kg (*p.o.*). When the

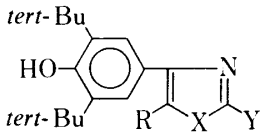
TABLE I. Anti-Inflammatory Activity of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-oxo or 2-Thioxoimidazolines



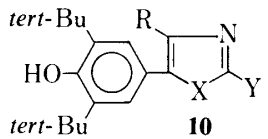
No.	R_1	X	Synth. method	Yield (%)	mp (°C)	Recryst. solvent	Formula		Analysis (%) Calcd (Found) C	H	N	A A[a] (25 mg/kg, *p.o.*) Therapeutic
6a	H	O	B	40	>260 (dec)	EtOH/H_2O	$C_{17}H_{24}N_2O_2$	1/2 C_2H_5OH	69.42 (69.08)	8.74 9.04	9.00 8.84)	IN
6b	CH_3	O	B	45.3	279—281 (dec)	EtOH/H_2O	$C_{18}H_{26}N_2O_2$		71.49 (71.47	8.67 8.82	9.26 9.22)	(+++)
6c	C_2H_5	O	B	47.9	267—270	Iso-PrOH/H_2O	$C_{19}H_{28}N_2O_2$		72.12 (71.83	8.92 9.17	8.85 8.62)	(+++)
6d	$(CH_3)_2CH$	O	B	19.5	>290 (dec)	Iso-PrOH/H_2O	$C_{20}H_{30}N_2O_2$		72.69 (72.37	9.15 9.26	8.48 8.47)	IN
6e	$(CH_3)_2CHCH_2$	O	B	60.7	286—289	C_6H_6/*n*-C_6H_{12}	$C_{21}H_{32}N_2O_2$		73.22 (73.43	9.36 9.57	8.13 8.18)	IN
6f	Cyclohexyl	O	B	32.5	>300	AcOH	$C_{23}H_{34}N_2O_2$	AcOH	69.74 (69.47	8.89 9.07	6.61 6.45)	IN
6g	C_6H_5	O	B	54.3	>300	DMF/H_2O	$C_{23}H_{28}N_2O_2$		75.79 (75.70	7.74 7.97	7.69 7.85)	IN
6h	H	S	C	57.8	168—170	C_6H_6/C_6H_{12}	$C_{17}H_{24}N_2OS$	1/2 C_6H_{12}	69.32 (69.33	8.73 8.46	8.08 7.76)	IN
6i	CH_3	S	C	57.8	288—290	Iso-PrOH	$C_{18}H_{26}N_2OS$	$(CH_3)_2CHOH$	66.63 (66.51	9.05 9.14	7.40 7.43)	IN
6j	C_2H_5	S	C	38.1	279—281	Iso-PrOH	$C_{19}H_{28}N_2OS$	$(CH_3)_2CHOH$	67.31 (67.17	9.24 9.36	7.14 7.29)	IN
6k	$(CH_3)_2CH$	S	C	21.4	>300	Iso-PrOH/H_2O	$C_{20}H_{30}N_2OS$		69.32 (69.18	8.73 8.95	8.08 7.74)	IN
6l	C_6H_5	S	C	41.8	258—262	Toluene	$C_{23}H_{28}N_2OS$		72.59 (72.70	7.42 7.52	7.36 7.49)	IN
Indomethacin (2 mg/kg, *p.o.*)												70—80%[b]

a) Adjuvant-induced arthritis. +++, therapeutic effect was larger than or equal to that of indomethacin; IN, inactive.
b) Inhibition (%) of edema formation induced by adjuvant.

TABLE II. Anti-Inflammatory Activity of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)imidazoles or -thiazoles



7—9 **11—14**



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No.	R	X	Y	Synth. method	Yield (%)	mp (°C)	Recryst. solvent	Formula		Analysis (%) Calcd (Found) C	H	N	A A[a] (25 mg/kg, *p.o.*) Therapeutic
7a	CH_3	NH	H	D	74.1	207—209	C_6H_{12}/*n*-C_6H_{14}	$C_{18}H_{26}N_2O$		75.48 (75.31	9.15 9.40	9.78 9.53)	(+)
7b	C_2H_5	NH	H	D	43.3	200—201	Toluene	$C_{19}H_{28}N_2O$	1/2 C_7H_8	77.99 (77.72	9.31 9.53	8.08 8.13)	(+)
7c	$(CH_3)_2CH$	NH	H	D	35.1	208—210	MeOH	$C_{20}H_{30}N_2O$	1/2 CH_3OH	74.50 (74.23	9.76 10.08	8.48 8.43)	N.T.
7d	C_6H_5	NH	H	D	43.0	170—171	Toluene	$C_{23}H_{28}N_2O$		79.27 (79.16	8.10 8.28	8.04 8.06)	IN
8a	H	NH	SCH_3	E	39.0	249—251	Toluene	$C_{18}H_{26}N_2OS$		67.89 (67.62	8.23 8.36	8.80 8.70)	IN
8b	CH_3	NH	SCH_3	E	30.0	184—186	C_6H_{12}	$C_{19}H_{28}N_2OS$		68.63 (68.42	8.49 8.74	8.43 8.42)	(+)
8c	CH_3	NH	SC_2H_5	E	47.5	245—250	Toluene	$C_{20}H_{30}N_2OS$		69.32 (69.38	8.73 8.70	8.08 8.00)	(±)
8d	CH_3	NH	$SCH(CH_3)_2$	E	30.4	250—258	Toluene	$C_{21}H_{32}N_2OS$		69.96 (69.74	8.95 9.02	7.77 7.61)	IN
8e	Iso-C_4H_9	NH	SCH_3	E	46.1	210—211	C_6H_6/*n*-C_6H_{14}	$C_{22}H_{34}N_2OS$		70.54 (70.45	9.15 9.24	7.48 7.17)	IN
8f	C_6H_5	NH	SCH_3	E	75.9	129—131	Toluene/ *n*-C_6H_{14}	$C_{24}H_{30}N_2OS$		73.06 (73.34	7.66 7.55	7.10 6.94)	IN
8g	C_2H_5	NH	SCH_3	E	60.0	210—211	Toluene	$C_{20}H_{30}N_2OS$		69.32 (69.05	8.73 8.68	8.08 7.68)	(++)
8h	C_2H_5	NH	SC_2H_5	E	28.8	226—228	Toluene	$C_{21}H_{32}N_2OS$		69.96 (70.18	8.95 8.97	7.77 7.63)	IN

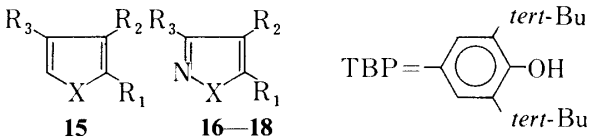
TABLE II. continued

No.	R	X	Y	Synth. method	Yield (%)	mp (°C)	Recryst. solvent	Formula		Analysis (%) Calcd (Found) C	H	N	A A[a] (25 mg/kg, *p.o.*) Therapeutic
8i	C_2H_5	NH	$SCH(CH_3)_2$	E	44.4	247—248	Toluene	$C_{22}H_{34}N_2OS$		70.54 (70.32	9.15 8.97	7.48 7.69)	IN
8j	CH_3	NH	SCH_2CF_3	E	18.4	228—229	C_6H_6	$C_{20}H_{27}F_3N_2OS$		59.98 (60.16	6.80 7.23	6.99 6.75)	IN
8k	CH_3	NH	SCF_2CF_2H	F	35.9	257—260	Toluene	$C_{20}H_{26}F_4N_2OS$		57.40 (57.91	6.26 6.49	6.69 6.83)	IN
8l	C_2H_5	NH	SCF_2CF_2H	F	21.5	245—247	Toluene	$C_{21}H_{28}F_4N_2OS$		58.32 (58.37	6.52 6.56	6.48 6.41)	IN
9a	H	NH	$SOCH_3$	G	38.7	106—108	C_6H_{12}	$C_{18}H_{26}N_2O_2S$	$1/2\,C_6H_{12}$	66.98 (66.44	8.57 8.33	7.44 7.41)	IN
9b	H	NH	SO_2CH_3	H	73.3	119—120	C_6H_{12}	$C_{18}H_{26}N_2O_3S$	$1/2\,C_6H_{12}$	64.25 (64.04	8.22 8.12	7.14 7.24)	IN
9c	CH_3	NH	SO_2CH_3	H	30.4	235—237	Toluene	$C_{19}H_{28}N_2O_3S$		62.61 (62.91	7.74 8.01	7.69 7.25)	IN
9d	CH_3	NH	$SO_2CF_2CF_2H$	H	39.8	248—250	Toluene	$C_{20}H_{26}F_4N_2O_3S$		53.32 (53.40	5.82 5.85	6.22 5.94)	IN
9e	CH_3	NH	SCH_2CO_2H	L	28.8	252—254	Iso-PrOH/H_2O	$C_{20}H_{28}N_2O_3S$		63.80 (63.53	7.50 7.74	7.44 7.26)	IN
10a	H	NCH_3	SH	I	18.8	288—289	EtOH	$C_{18}H_{26}N_2OS$		67.89 (67.67	8.23 8.50	8.80 8.56)	IN
10b	CH_3	NCH_3	OH	I	42.9	285—286	Iso-PrOH/H_2O	$C_{19}H_{28}N_2O_2$		72.12 (72.18	8.92 9.18	8.85 8.57)	IN
11a	H	S	SH	J	56.7	285—288	Dioxane	$C_{17}H_{23}NOS_2$	$1/2C_4H_8O_2$	62.43 (62.31	7.44 7.41	3.83 3.69)	(±)
11b	CH_3	S	SH	J	71.4	267—269	THF/*n*-C_6H_{14}	$C_{18}H_{25}NOS_2$		64.44 (64.29	7.51 7.50	4.17 4.16)	IN

11c	C_6H_5	S	SH	J	72.9	168—171	THF/*n*-C_6H_{14}	$C_{23}H_{27}NOS_2$	69.48 (69.80	6.48 7.10	3.52 3.43)	IN
12a	H	S	SCH_3	K	73.0	92—93	*n*-C_6H_{14}	$C_{18}H_{25}NOS_2$	64.44 (64.36	7.51 7.69	4.17 4.23)	(++)
12b	H	S	SC_2H_5	K	71.1	130—131	MeOH/H_2O	$C_{19}H_{27}NOS_2$	65.29 (64.97	7.79 7.99	4.01 4.01)	IN
12c	H	S	$SCH(CH_3)_2$	K	70.0	173—174	*n*-C_6H_{14}	$C_{20}H_{29}NOS_2$	66.07 (66.20	8.04 7.84	3.85 3.67)	N.T.
12d	H	S	SCF_2CF_2H	F	44.8	88—89	Iso-PrOH/H_2O	$C_{19}H_{23}F_4NOS_2$	54.14 (54.14	5.50 5.61	3.32 3.45)	IN
12e	H	S	SCH_2CO_2H	L	71.8	158—159	C_6H_6/*n*-C_6H_{14}	$C_{19}H_{25}NO_3S_2$	60.13 (60.06	6.64 6.62	3.69 3.77)	N.T.
12f	CH_3	S	SCH_3	K	72.3	118—119	*n*-C_6H_{14}	$C_{19}H_{27}NOS_2$	65.29 (65.41	7.79 7.96	4.01 4.01)	IN
12g	C_2H_5	S	SCH_3	K	63.9	115—116	*n*-C_6H_{14}	$C_{20}H_{29}NOS_2$	66.07 (66.17	8.04 8.18	3.85 4.11)	IN
12h	$CH(CH_3)_2$	S	SCH_3	K	77.1	135—137	MeOH	$C_{21}H_{31}NOS_2$	66.80 (66.70	8.27 8.37	3.71 3.77)	IN
12i	C_6H_{11}	S	SCH_3	K	73.5	168—169	EtOH	$C_{24}H_{35}NOS_2$	69.02 (68.74	8.45 8.69	3.35 3.32)	IN
12j	C_6H_5	S	SCH_3	K	66.3	135—137	*n*-C_6H_{14}	$C_{24}H_{29}NOS_2$	70.03 (70.30	7.10 7.37	3.40 3.44)	IN
13a	H	S	$SOCH_3$	G	53.8	124—125	*n*-C_6H_{14}	$C_{18}H_{25}NO_2S_2$	61.50 (61.41	7.17 7.32	3.98 4.05)	IN
13b	H	S	SO_2CH_3	H	62.5	183—184	MeOH	$C_{18}H_{25}NO_3S_2$	58.83 (58.72	6.86 7.00	3.81 3.69)	IN
14a	CH_3	S	H	R[b]	39.7	128—130	C_6H_{12}/*n*-C_6H_{14}	$C_{18}H_{25}NOS$	71.24 (71.39	8.30 8.53	4.62 4.38)	IN
14b	CH_3	O	H	R[b]	58.1	105—107	MeOH/H_2O	$C_{18}H_{25}NO_2$	75.23 (75.07	8.77 8.91	4.87 4.68)	IN
Indomethacin (2 mg/kg, *p.o.*)												70—80%[c]

a) Adjuvant-induced arthritis. ++, therapeutic effect was less than that of indomethacin; +, therapeutic effect was less than half that of idomethacin; ±, therapeutic effect was noted but was not significant; IN, inactive; N.T., not tested.
b) See Experimental section.
c) Inhibition (%) of edema formation induced by adjuvant.

TABLE III. Anti-Inflammatory Activity of 4-Pyrrole, 4-Pyrazole and 4-Isoxazole-substituted 2,6-Di-*tert*-butylphenols



15 **16—18**

No.	X	R_1	R_2	R_3	Synth. method	Yield (%)	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	A A[a] (25 mg/kg, *p.o.*) Therapeutic
15	NH	CH_3	TBP	H	R[c]	40.2	118—120	*n*-C_6H_{14}	$C_{19}H_{27}NO$	79.95 (80.09	9.53 9.89	4.91 4.76)	IN
16	NH	TBP	CH_3	CH_3S	R[c]	54.8	198—199	C_6H_{12}	$C_{19}H_{28}N_2OS$	68.63 (68.80	8.49 8.79	8.43 8.46)	IN
17	NH	TBP	CH_3	CH_3SO	G	76.3	206—208	Toluene/ *n*-C_6H_{14}	$C_{19}H_{28}N_2O_2S$	65.48 (65.45	8.10 8.25	8.04 7.93)	IN
18	O	TBP	CH_3	H	R[c]	13.9	129—130	*n*-C_6H_{14}	$C_{18}H_{25}NO_2$	75.23 (75.69	8.77 9.13	4.87 4.37)	(+)
Indomethacin (2 mg/kg, *p.o.*)													70—80%[b]

a) Adjuvant-induced arthritis. +, therapeutic effect was less than half that of indomethacin; IN, inactive.
b) Inhibition (%) of edema formation induced by adjuvant.
c) See Experimental section.

TABLE IV. Summary of Biological Properties

Compd. No.	Adjuvant arthritis (25 mg/kg, *p.o.*) Therapeutic $\lvert\Delta FT\rvert(10^{-2}$ mm)[a] Test compd/IM[d]	Prophylactic (%)[b] FT_L	FT_R	C.I.P.E.[c] (25 mg/kg, *p.o.*) (%)	Analgesic[i] (50 mg/kg, *p.o.*) (%)	Acute toxicity (*p.o.*)
6b	216±30 /173±62	64.6	81.8	39.1	25.0	>1000 mg/kg, mouse >400 mg/kg, rat
6c	247±49 /204±33	N.T.[e]	N.T.	37.9	21.4	
7a	122±46[f]/216±30	47.5	69.6	42.9	67.0	
7b	103±71[g]/204±33	N.T.	N.T.	30.8	42.6	
8b	153±56[f]/216±30	59.5	76.9	38.0	27.0	
8c	69±51[h]/161±56	N.T.	N.T.	N.T.	N.T.	
8g	180±98[f]/161±56	N.T.	N.T.	N.T.	N.T.	
11a	57±26[g]/204±33	N.T.	N.T.	2.9[h]	N.T.	
12a	166±59 /204±33	N.T.	N.T.	25.6	12.2[h]	
18	65±33[f]/134±50	N.T.	N.T.	N.T.	N.T.	
Indomethacin		68.1	81.3	44.0 (3 mg/kg)	N.T.	
Aminopyrine					63.0	
Phenylbutazone				33.2 (50 mg/kg)		

a) The change of foot thickness was calculated as the difference between the values on days 15 and 28. $p<0.01$ *vs.* control.
b) Inhibition (%) of edema formation. $p<0.01$ *vs.* control.
c) Carrageenin-induced rat paw edema, inhibition (%) of edema formation. $p<0.001$ *vs.* control.
d) IM: indomethacin (2 mg/kg, *p.o.*).
e) NT: not tested.
f) $p<0.05$ *vs.* arthritis control.
g) $p<0.01$ *vs.* arthritis control.
h) Not significant.
i) Inhibition (%) of writhing induced by AcOH in mice. $p<0.001$ *vs.* control.

oxygen atom at the 2-position of **6b** was replaced with a sulfur atom, which is an isostere (compound **6i**), no activity was seen, but with S-alkyl analogues of **6i**, compounds **8b** and **8c** showed activity and compound **8d** which has a bulky alkylthio group did not show any activity. When the oxygen atom of **6b** was replaced with a hydrogen atom (compound **7a**), the potency of anti-inflammatory activity decreased. The N-methylated compound **10b** showed no activity either. These results indicated that the steric factor is important for the substituent at the 5-position of **6a** and that the 2-position of **6b** is rather critical. Among the compounds with other heterocycles which have a steric interaction between the 2,6-di-*tert*-butylphenol group and the methyl group similar to that in **7a** (compounds **14a**, **14b**, **15** and **18**), compound **18** showed very weak activity while the others showed none. With thiazole compounds, **11a** showed weak activity.

In contrast with the result of the modification of **6a**, compound **12a** showed potent activity. However, compounds which have a substituent at the 5-position of **12a** (compounds **12f—j**) did not show any activity and neither did compounds which have a large alkylthio group at the 2-position (compounds **12b—e**). From these facts, the anti-inflammatory activity of 2,6-di-*tert*-butylphenols appeared to depend on the heterocyclic group in the 4-position, as described in an earlier paper.[1] Compounds **6b**, **6c**, and **12a** were further tested in adjuvant-induced arthritis (prophylactic activity) assay, carrageenin-induced rat paw edema assay and AcOH-induced writhing assay in mice. The results are listed in Table IV. Compound **6b** showed strong activity in these tests and had low toxicity (acute toxicity >1000 mg/kg, *p.o.*, mouse and >400 mg/kg, *p.o.*, rat). Compound **6b** had minimum effective dose of 6.25 mg/kg

(*p.o.*) in AA assay (therapeutic). Compound **6b** (25 mg/kg, *p.o.*) had about the same activity as indomethacin (2 mg/kg, *p.o.*) in AA assay (prophylactic), 39.1% inhibition of edema formation in carrageenin-induced rat paw edema assay (25 mg/kg, *p.o.*) and 25% inhibition of writhing in AcOH-induced writhing assay (50 mg/kg, *p.o.*). It is well known that there is a possibility that the active compounds in the rat foot edema model are acting merely by stimulating the adrenals. So, in order to eliminate this possibility, **6b** was tested in carrageenin-induced edema in adrenalectomized rats. The potency of **6b** did not decrease. Compound **6b** showed radical-scavenging activity *in vivo* and *in vitro* and mild inhibitory activity of delayed-type hypersensitivity. From these results, compound **6b** is considered to be a promising candidate for a new anti-arthritic agent. These pharmacological data will be published elsewhere. A detailed pharmacological study of **6b** is in progress.

Experimental

All melting points were determined by using a Yanagimoto micromelting point apparatus and are uncorrected. Infrared (IR) spectra were obtained with a Hitachi 215 spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained with a JEOL-MH 100 or a JEOL-FX 90 spectrometer with $(CH_3)_4Si$ as an internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra (MS) were obtained with an RMU-6MG spectrometer and the abbreviation RI means relative intensity. The spectral data for all new compounds were consistent with their structure.

Preparation of 4-(2-Aminoacyl)-2,6-di-*tert*-butylphenols (5a—g) (Method A): 4-(2-Aminoacetyl)-2,6-di-*tert*-butylphenol Hydrochloride (5a)——A solution of 0.65 g (10 mmol) of sodium azide in 5 ml of H_2O was added dropwise to a solution of 3.27 g (10 mmol) of 4-(2-bromoacetyl)-2,6-di-*tert*-butylphenol in 20 ml of acetone at room temperature. After being stirred for 1 h, the reaction mixture was poured into H_2O and extracted with AcOEt.

The extract was dried and concentrated *in vacuo*. The residue was dissolved in 20 ml of methanol and 0.5 ml of CHCl was added. It was reduced by catalytic hydrogenation on Pd-C as a catalyst.

After the reduction, the catalyst was removed by filtration and the filtrate was evaporated to dryness *in vacuo* to give **5a**, 2.0 g. Compounds **5a—f** were not purified, but **5b** melted at 208—210 °C (from MeOH–Et_2O); *Anal.* Calcd for $C_{17}H_{28}ClNO_2$: C, 65.06, H, 8.99, N 4.46; Found: C 64.94, H 8.91, N 4.43.

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-oxo-4-imidazolines (6a—d) (Method B): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-oxo-4-imidazoline (6a)——A solution of potassium isocyanate (0.64 g, 10 mmol) in H_2O (3 ml) was added dropwise to a mixture of 4-(2-aminoacetyl)-2,6-di-*tert*-butylphenol hydrochloride (1.20 g, 4 mmol), ethanol (10 ml) and CHCl (0.3 ml) at room temperature. The mixture was stirred for 2 h, then CHCl (0.3 ml) was added. The whole was heated under reflux for 2 h. After being cooled to room temperature, it was poured into H_2O (30 ml) and the resulting precipitate was collected by filtration. The precipitate was recrystallized from aqueous ethanol to give **6a** (0.50 g, 40%): IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 288 (M^+, RI = 100%); NMR (DMSO-d_6) δ: 1.08 (1.5H, t, J = 6 Hz, CH_3CH_2), 1.36 (18H, s, $(C\underline{H}_3)_3C$), 3.42 (1H, q, J = 6 Hz, $CH_3C\underline{H}_2$), 6.54 (1H, s, C_5–H), 6.82 (1H, s, phenolic-OH), 7.12 (2H, s, aromatic-H), 9.72 (1H, s, NH), 10.32 (1H, s, NH). Compound **6f**: MS *m/z*: 370 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.20—1.60 (24H, br, $(CH_3)_3C$, cyclohexyl-H), 1.60—1.90 (4H, br, cyclohexyl-H), 2.20 (3H, s, CH_3COOH), 2.70 (1H, br, cyclohexyl-H), 5.20 (1H, s, OH), 7.10 (2H, s, aromatic-H), 10.10 (1H, s, NH), 10.50 (1H, s, NH).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-thioxo-4-imidazolines (6h—l) (Method C): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-thioxo-4-imidazoline (6h)——A solution of sodium thiocyanate (9.72 g, 120 mmol) in H_2O (20 ml) was added dropwise to a mixture of 4-(2-aminoacetyl)-2,6-di-*tert*-butylphenol hydrochloride (18.0 g, 60 mmol), ethanol (150 ml) and CHCl (5 ml) at room temperature. After being stirred for 1.5 h at room temperature, the mixture was heated under reflux for 2 h and then concentrated *in vacuo*. The residue was dissolved in AcOH (100 ml) and the solution was heated for 2—3 h under reflux. After being cooled to room temperature, it was poured into H_2O and the resulting precipitate was collected by filtration. The precipitate was recrystallized from benzene–cyclohexane to give **6h** (12.0 g, 57.8%): IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 304 (M^+, RI = 64%); NMR ($CDCl_3$) δ: 1.44 (24H, s, $(CH_3)_3C$, cyclohexyl-H), 5.32 (1H, s, OH), 6.78 (1H, s, C_5–H), 7.24 (2H, s, aromatic-H), 12.2 (2H, br, NH).

Compounds **6e—g** were prepared in a similar manner.

Compound **6i**: MS *m/z*: 318 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.20 (6H, d, J = 6 Hz, $(CH_3)_2C$), 1.40 (18H, s, $(CH_3)_3C$), 2.00 (1H, br, OH), 4.00 (1H, quintet, J = 6 Hz, $(CH_3)_2C\underline{H}$), 5.30 (1H, s, OH), 7.16 (2H, s, aromatic-H), 11.20 (1H, br, NH), 11.80 (1H, br, NH). Compound **6j**: MS *m/z*: 332 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.20 (6H, d, J = 6 Hz, $(C\underline{H}_3)_2CH$), 1.22 (3H, t, J = 7 Hz, $C\underline{H}_3CH_2$), 1.42 (18H, s, $(CH_3)_3C$), 2.60 (2H, q, J = 7 Hz, $CH_3C\underline{H}_2$), 4.00 (1H, quintet, J = 6 Hz, $(CH_3)_2C\underline{H}$), 5.30 (1H, s, OH), 7.10 (2H, s, aromatic-H), 10.45 (1H, br, NH), 11.10 (1H, br,

NH).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)imidazoles (7a—e) (Method D): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-methylimidazole (7a)——A mixture of **6i** (1.50 g, 4.7 mmol), Raney nickel (0.50 g) and absolute ethanol (50 ml) was heated under reflux for 1 h, then filtered. The filtrate was concentrated *in vacuo*. The residue was recrystallized from cyclohexane–hexane to give **7a** (1.0 g, 74.1%): IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 286 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 2.40 (3H, s, C_5–CH_3), 6.04 (1H, br, OH), 7.32 (2H, s, aromatic-H), 7.50 (1H, s, C_2–H).

Compound **7b**: MS *m/z*: 300 (M^+, RI = 100%); NMR ($CDCl_3$): 1.28 (3H, t, J = 7 Hz, CH_3CH_2), 1.41 (18H, s, $(CH_3)_3C$), 2.32 (1.5H, s, CH_3–C_6H_5), 2.78 (2H, q, J = 7 Hz, CH_3CH_2), 5.20 (1H, br, OH), 7.10 (2H, s, aromatic-H), 7.30 (2.5H, s, aromatic-H), 7.48 (1H, s, C_2–H). Compound **7c**: MS *m/z*: 314 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.30 (6H, d, J = 6 Hz, $(CH_3)_2CH$), 1.40 (18H, s, $(CH_3)_3C$), 3.20 (1H, quintet, J = 6 Hz, $(CH_3)_2CH$), 3.42 (1.5H, s, CH_3OH), 5.20 (1H, br, OH), 7.22 (2H, s, aromatic-H), 7.42 (1H, s, C_2–H).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-alkylthioimidazoles (8a—j) (Method E): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methylthioimidazole (8a)——Methyl iodide (1.42 g, 10 mmol) was added dropwise to a mixture of **6h** (3.0 g, 10 mmol), dry acetone (50 ml) and K_2CO_3 (1.30 g, 10 mmol) at room temperature. The mixture was stirred for 1 h, then the solvent was evaporated off *in vacuo*. H_2O (50 ml) was added to the residue and the resulting precipitate was collected by filtration to give crude product (2.0 g). The crude product (0.80 g) was recrystallized from toluene to afford **8a** (0.50 g, 39%): IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 318 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.40 (18H, s, $(CH_3)_3C$), 2.54 (3H, s, S–CH_3), 5.20 (1H, br, OH), 7.16 (1H, s, NH or C_5–H), 7.22 (1H, s, NH or C_5–H), 7.36 (2H, s, aromatic-H).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(1,1,2,2-tetrafluoroalkylthio)imidazoles (8k—l) (Method F): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-methyl-2-(1,1,2,2-tetrafluoroethylthio)imidazole (8k)——Sodium (40 mg, 1.74 mmol) was added to a solution of **6i** (3.16 g, 10 mmol) in dimethylformamide (DMF, 20 ml) at room temperature. The solution was cooled below −50 °C and a solution of tetrafluoroethylene (2.0 g, 20 mmol) in DMF (50 ml) was added dropwise to it. Then the temperature of the reaction mixture was increased gradually to 100 °C and kept there for 20 min. After being cooled, the reaction mixture was poured into dil. HCl. The resulting precipitate was collected, applied to a column of silica gel, and eluted with $CHCl_3$. The product was recrystallized from toluene to give **8k** (1.50 g, 35.9%): MS *m/z*: 418 (M^+, RI = 100%); NMR (DMSO-d_6) δ: 1.40 (18H, s, $(CH_3)_3C$), 2.32 (3H, s, C_5–CH_3), 6.08 (1H, t, J = 3.6 Hz, CF_2HCF_2H), 6.64 (1H, t, J = 3.6 Hz, CF_2HCF_2H), 6.92 (1H, br, OH), 7.28 (2H, s, aromatic-H). Compound **8e** was prepared in a manner similar to that described for **8k**.

4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methylsulfinylimidazole (9a) (Method G)——*m*-Chloroperbenzoic acid (0.18 g, 1.15 mmol) was added portionwise to a solution of **8a** (0.32 g, 1 mmol) in $CHCl_3$ (5 ml) at room temperature. The reaction mixture was stirred overnight and then washed with dil. $NaHCO_3$.

The organic layer was dried and concentrated *in vacuo*. The residue was recrystallized from cyclohexane to give **9a** (0.13 g, 38.7%); IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 334 (M^+, RI = 21%); NMR ($CDCl_3$) δ: 1.40 (6H, s, cyclohexyl-H), 1.48 (18H, s, $(CH_3)_3C$), 3.10 (3H, s, CH_3), 5.30 (1H, s, OH), 7.32 (1H, s, C_5–H), 7.44 (2H, s, aromatic-H).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-alkylsulfonylimidazoles (9b—d) and -thiazole (13b) (Method H): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methylsulfonylimidazole (9b)——A mixture of **8a** (0.62 g, 2 mmol), *m*-chloroperbenzoic acid (0.44 g, 2.8 mmol) and 1,2-dimethoxyethane (10 ml) was heated under reflux for 3—4 h. The reaction mixture was washed with dil. $NaHCO_3$ and concentrated *in vacuo*. The residue was recrystallized from cyclohexane to give **9b** (0.50 g, 73.3%); IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 350 (M^+, RI = 20%); NMR ($CDCl_3$) δ: 1.40 (24H, s, $(CH_3)_3C$, cyclohexyl-H), 3.06 (3H, s, SO_2CH_3), 5.06 (1H, s, OH), 7.30 (1H, s, C_5–H), 7.48 (2H, s, aromatic-H).

Preparation of 5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-methylimidazoles (10a, b) (Method I): 5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-mercapto-1-methylimidazole (10a)——A mixture of 4-(2-aminoacetyl)-2,6-di-*tert*-butylphenol hydrochloride (3.0 g, 10 mmol), methyl thiocyanate (6.0 g, 82 mmol) and pyridine (100 ml) was stirred for 2 h at room temperature, then the temperature of the reaction mixture was increased to 80—90 °C and kept there for 1.5 h. After being cooled to room temperature, the mixture was concentrated *in vacuo* and extracted with AcOEt (100 ml). The extract was washed with dil. HCl, dried and evaporated *in vacuo*. The residue was recrystallized from ethanol to give **10a** (0.60 g, 18.8%): MS *m/z*: 318 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 3.54 (3H, s, N–CH_3), 6.64 (1H, s, C_4–H), 7.08 (2H, s, aromatic-H).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-mercaptothiazoles (11a—c) (Method J): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-mercaptothiazole (11a)——4-(2-Bromoacetyl)-2,6-di-*tert*-butylphenol (15.0 g, 45.9 mmol) was added portionwise to a suspension of ammonium dithiocarbamate (10.0 g, 91 mmol) in ethanol (60 ml) at 0—5 °C. The mixture was stirred overnight at room temperature, then H_2O (200 ml) was added and the whole was extracted with benzene. The extract was dried and concentrated *in vacuo*. The residue was dissolved in AcOH (50 ml) and the solution was heated under reflux for 4 h. After being cooled to room temperature, it was poured into a mixture of AcOEt (50 ml) and H_2O (150 ml). The resulting precipitate was collected by filtration and recrystallized from dioxane to give **11a** (9.5 g, 56.7%); MS *m/z*: 321 (M^+, RI = 100%); NMR (DMSO-d_6) δ: 1.42 (18H, s, $(CH_3)_3C$),

3.28 (4H, s, dioxane-H), 7.0 (1H, s, C_5–H), 7.24 (1H, s, OH), 7.34 (2H, s, aromatic-H), 10.48 (1H, br, SH).

Preparation of 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-alkylthiazoles (12a—c, 12f—j) (Method K): 4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methylthiothiazole (12a)——Sodium (0.18 g, 7.8 mmol) was dissolved in methanol (30 ml) and **11a** (2.1 g, 6.5 mmol) was added portionwise to it. The reaction mixture was cooled to 0—5 °C and methyl iodide (1.15 g, 8.1 mmol) was added dropwise. The mixture was stirred for 0.5 h, then H_2O (60 ml) was added. The resulting precipitate was collected and recrystallized from *n*-hexane to give **12a** (1.6 g, 73%); MS *m/z*: 335 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 2.72 (3H, s, SCH_3), 5.24 (1H, s, OH), 7.10 (1H, s, C_5–H), 7.62 (2H, s, aromatic-H).

Preparation of the Thioacetic Acids (9e and 12e) (Method L): [4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-thiazolylthio]acetic Acid (12e)——Triethylamine (0.50 g, 5 mmol) was added dropwise to a mixture of **11a** (0.64 g, 2 mmol), α-bromoacetic acid (0.32 g, 2.3 mmol) and benzene (20 ml) under stirring. After 1 h, the resulting precipitate was filtered off. The filtrate was extracted with 2% NaOH (10 ml). The extract was acidified with dil. HCl and extracted with Et_2O. The extract was dried and concentrated *in vacuo*. The residue was recrystallized from benzene to give **12e** (0.52 g, 71.8%); MS *m/z*: 379 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 3.84 (2H, s, SCH_2), 5.40 (1H, s, OH), 7.20 (1H, s, C_5–H), 7.52 (2H, s, aromatic-H).

4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-methylthiazole (14a)——A mixture of thioformamide (0.70 g, 11 mmol), 4-(2-bromopropionyl)-2,6-di-*tert*-butylphenol (3.4 g, 10 mmol) and absolute ethanol (15 ml) was heated at 50—60 °C for 2—3 h. After being cooled to room temperature, it was poured into dil. K_2CO_3.

The resulting precipitate was collected and recrystallized from cyclohexane–*n*-hexane to give **14a** (1.20 g, 39.7%); MS *m/z*: 303 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 2.56 (3H, s, C_5–CH_3), 5.14 (1H, s, OH), 7.40 (2H, s, aromatic-H), 8.36 (1H, s, C_2–H).

4-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-methyloxazole (14b)——A mixture of formamide (4.50 g, 0.1 mol) and 4-(2-bromopropionyl)-2,6-di-*tert*-butylphenol (2.25 g, 6.6 mmol) was heated at 150 °C for 1.5 h. It was poured into cold H_2O (100 ml) and extracted with benzene (30 ml) twice. The extract was dried and concentrated *in vacuo*. The residue was recrystallized from aqueous methanol to give **14b** (1.10 g, 58.1%); IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 287 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 2.50 (3H, s, C_5–CH_3), 5.22 (1H, s, OH), 7.42 (2H, s, aromatic-H), 7.70 (1H, s, C_2–H).

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methylpyrrole (15)——A mixture of 4-(2-aminopropionyl)-2,6-di-*tert*-butylphenol hydrochloride (9.40 g, 30 mmol), acetylene dicarboxylic acid dimethyl ester (4.20 g, 30 mmol), sodium acetate (2.46 g, 30 mmol) and methanol (75 ml) was heated under reflux for 2 h and then concentrated *in vacuo*. The residue was extracted with $CHCl_3$ (50 ml) twice and the extract was washed with H_2O, dried and evaporated *in vacuo*. The residue was applied to a column of silica gel and eluted with $CHCl_3$ to give crude product (2.0 g). A mixture of this crude product (0.5 g, 1.2 mmol), lithium iodide (0.78 g, 5.8 mmol), and collidine (10 ml) was heated under reflux until evolution of CO_2 ceased, then it was poured into H_2O and the resulting precipitate was collected by filtration. The precipitate was applied to a column of silica gel, eluted with $CHCl_3$ and recrystallized from *n*-hexane to give **15** (0.10 g, 40.2%); IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 285 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 2.36 (3H, s, C_2–CH_3), 5.04 (1H, s, OH), 6.24 (1H, t, J = 4 Hz, C_4–H), 6.62 (1H, t, J = 4 Hz, C_5–H), 7.16 (2H, s, aromatic-H), 7.80 (1H, br, NH).

5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-4-methyl-3-methylthiopyrazole (16)——Hydradine carbodithioic acid methyl ester (2.52 g, 20 mmol) was added to a solution of sodium (0.46 g, 20 mmol) in absolute ethanol (40 ml) below 10 °C. The mixture was stirred for 15 min, then 4-(2-bromopropionyl)-2,6-di-*tert*-butyl-phenol (6.82 g, 20 mmol) was added portionwise to the mixture below 10 °C, and the whole was stirred for 2 h at room temperature. Then the solvent was evaporated off *in vacuo*. A mixture of AcOEt (50 ml) and H_2O (50 ml) was added to the residue and the organic layer was separated. It was dried and concentrated *in vacuo*. The residue was recrystallized from *n*-hexane to give 5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-6-methyl-2-methylthio-6*H*-1,3,4-thiadiazine (4.0 g, 54.9%), mp 151—152 °C. This product (3.0 g, 8.2 mmol) was dissolved in AcOH (30 ml), and the solution was heated under reflux for 0.5 h. After being cooled to room temperature, it was poured into H_2O (100 ml). The resulting precipitate was collected by filtration and recrystallized from *n*-hexane to give **16** (1.50 g, 54.8%); IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 332 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.44 (18H, s, $(CH_3)_3C$), 2.16 (3H, s, C_4–CH_3), 2.44 (3H, s, SCH_3), 5.20 (1H, br, OH), 7.26 (2H, s, aromatic-H). Compound **17** was prepared in a manner similar to that described for **9a**.

5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-4-methylisoxazole (18)——A mixture of 3,5-di-*tert*-butyl-4-hydroxypropiophenone (2.62 g, 10 mmol) and *N,N*-dimethylformamide diethylacetal (15 ml) was heated under reflux for 4 h and then concentrated *in vacuo*. The residue was dissolved in absolute methanol (20 ml) and the solution was cooled to 0 °C. Hydroxylamine-*O*-sulfonic acid (1.24 g, 11 mmol) was added to it portionwise and the whole was stirred for 1 h. The solvent was evaporated off *in vacuo*, and the residue was extracted with AcOEt (50 ml). The extract was dried and evaporated *in vacuo*. The residue was applied to a column of silica gel, eluted with $CHCl_3$ and recrystallized from *n*-hexane to give **18** (0.50 g, 13.9%); IR (KBr): 3600 (OH) cm^{-1}; MS *m/z*: 287 (M^+, RI = 100%); NMR ($CDCl_3$) δ: 1.48 (18H, s, $(CH_3)_3C$), 2.24 (3H, s, C_4–CH_3), 5.48 (1H, s, OH), 7.56 (2H, s, aromatic-H), 8.10 (1H, s).

Biological Test Procedures——1) Carrageenin-Induced Rat Paw Edema Assay: Inhibitory activity of these

compounds on edema formation was determined by means of the carrageenin-induced rat paw edema assay as described by Winter *et al.*[14] Male Wistar rats (140—160 g) were placed in groups of 6 animals. Test compounds were administered orally as an aqueous suspension and the phlogistic agent (0.1 ml of 1% carrageenin in 0.9% sterile sodium chloride) was injected 1 h later into the plantar area of the left hind paw of each animal. The animals were sacrificed by administration of $CHCl_3$ 3 h later, and then the hind paws were amputated at the tibiotarsal joint and weighed. The difference in weight between injected and noninjected paws was recorded and a statistical analysis of the data was carried out. The activity recorded in Table IV refers to the oral screening dose.

2) Adjuvant-Induced Arthritis Assay: The activity was determined according to the method described by Pearson.[15] Male Sprague Dawley rats (aged 7 weeks) were placed in groups of 3 or 6 animals. On day 0, heat-killed *Mycobacterium butyricum* was suspended in light mineral oil (600 μg/0.1 ml) and injected into the left paw of each rat. Test compounds wee administered therapeutically (days 15—27) or prophylactically (days 0—21). In the therapeutic assay, all the animals were evaluated on day 15 and arthritic rats were selected for use. These animals were randomly arranged into groups of 3 or 6 and treated through days 15—27. The thickness of the foot was measured with a dial thickness gauge on days 15 and 28 (therapeutic assay) or days 4 and 21 (prophylactic assay). The change of foot thickness was calculated as the difference between the values on day 15 and day 28 (therapeutic assay). All the agents were administered as an aqueous suspension at constant volume (5 ml/kg/rat).

3) Analgesic Activity: Activity was evaluated by the AcOH-induced writhing assay described by Koster *et al.*[16] Male ICR mice weighing 25—32 g which had been fasted overnight were used; each group consisted of 8—10 animals. Thirty min after oral administration of test drugs (0.5% carboxymethyl cellulose suspension), 0.1 ml/10 g body weight of 0.6% AcOH solution was injected into the peritoneal cavity. The frequency of writhing was then counted in each animal for 20 min. The response of the drug-treated mice was compared with the response of those given AcOH alone.

4) Acute Toxicity: Male ICR mice weighing about 30 g and Wistar rats weighing about 150 g were used. The animals ($n=5$) had free access to food and water during the test period. Compound **6b** was administered to mice at doses of 500 and 1000 mg/kg (*p.o.*), and to rats at doses of 100, 200 and 400 mg/kg (*p.o.*). The behavior of each animal was observed for 7 d. After 7 d, the body weight gain was compared with that of the control animals. No abnormality in the behavior of any animal and no significant difference in body weight gain between the treated groups and the control group were noticed.

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