centrated. The residual red oil slowly crystallized and was recrystallized from methanol to give 17.7 g (41%) of the diester. A second crystallization from methanol gave pure **18a**: mp 54–58 °C (lit.[32] mp 59 °C). Anal. ($C_{15}H_{15}NO_5$) C, H, N.

The oxazole diester **18a** (11.7 g, 40 mmol) in anhydrous ether (150 mL) was added dropwise to lithium aluminum hydride (3.84 g, 101 mmol) in refluxing anhydrous ether. The reaction was refluxed 1 h and cooled and then water (3.8 mL), 10% sodium hydroxide (3.8 mL), and water (11.4 mL) were added. The resulting salts were extracted with ether for 26 h in a Soxhlet apparatus, the ether was removed, and the residue was crystallized from ethyl acetate to give 3.3 g (40%) of **18b**: mp 127–129 °C; NMR (Me_2SO-d_6/Me_4Si) δ 4.52–4.70 (m, 4 H), 5.27 (br s, 3 H), 7.50–8.07 (m, 6 H); IR (KBr) 3575, 3425, 1650, 1630, 1580, 1500, 1460, 1030, 720 cm^{-1}. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

2-Phenyl-4,5-bis(hydroxymethyl)oxazole Bis(methylcarbamate) (18c). The oxazole diol **18b** (3.18 g, 15.5 mmol), methyl isocyanate (5.6 mL, 94.9 mmol), and triethylamine (1 mL) were heated at reflux for 3 h in dichloromethane (125 mL). The suspension cleared after 10 min, and within 40 min, a precipitate occurred. The reaction was stirred an additional 9 h at room temperature. The precipitate was filtered and dried under a stream of nitrogen and then under high vacuum to give 3.93 g, mp 190–192 °C. The filtrate was concentrated and crystallization of the residue from ethyl acetate gave an additional 0.63 g for a total yield of **18c** of 92%: NMR (Me_2SO-d_6/Me_4Si) δ 2.57 (s), 2.65 [s, 6 H (total for both singlets)], 5.03 (s, 2 H), 5.22 (s, 2 H), 7.1 (br, 2 H), 7.47–8.03 (m, 5 H); NMR ($CDCl_3$/Me_4Si) δ 2.77 (d, J = 1 Hz), 2.84 [d, J = 1 Hz, 6 H (total for both doublets)], 4.9 (br, 2 H), 5.16 (s), 5.29 [s, 4 H (total for both singlets)], 7.40–8.10 (m, 5 H); IR (KBr) 3350, 2975, 1700, 1550, 1490, 1470, 1450, 1430, 1350, 1290, 1270, 1260, 1140, 1070, 990, 960, 780, 720 cm^{-1}. Anal. ($C_{15}H_{17}N_3O_5$) C, H, N.

Acknowledgment. This investigation was supported by Public Health Service Grant R01-CA-22935 awarded by the National Cancer Institute, National Institutes of Health, DHHS.

Registry No. **1a**, 66981-69-9; **1b**, 92126-11-9; **2a**, 28682-57-7; **2b**, 92126-12-0; **3a**, 92126-08-4; **3b**, 92126-13-1; **4a**, 92126-06-2; **4b**, 92126-09-5; **5**, 92126-14-2; **6**, 92126-16-4; **7**, 92126-17-5; **8a**, 28317-50-2; **8b**, 27143-09-5; **8c**, 28663-68-5; **8d**, 92126-02-8; **9a**, 92126-18-6; **9b**, 92126-19-7; **9c**, 7189-00-6; **9d**, 92126-03-9; **10a**, 92126-20-0; **10b**, 92126-21-1; **10c**, 92126-22-2; **10d**, 92126-04-0; **11a**, 92126-23-3; **11b**, 92126-24-4; **11c**, 92126-25-5; **11d**, 92126-05-1; **12**, 92126-26-6; **13a**, 20851-16-5; **13b**, 92126-27-7; **13c**, 92126-28-8; **14a**, 17304-69-7; **14b**, 51808-10-7; **14c**, 92126-29-9; **15a**, 7710-44-3; **15b**, 92126-30-2; **15c**, 92126-31-3; **16a**, 27545-53-5; **16b**, 92126-32-4; **16c**, 92126-33-5; **17a**, 24044-79-9; **17b**, 92126-34-6; **17c**, 92126-35-7; **18a**, 15926-46-2; **18b**, 92126-36-8; **18c**, 92126-37-9; **19**, 825-60-5; 19·HCl, 5333-86-8; DMAD, 762-42-5; $(CH_3)_2S$, 75-18-3; $PhCOCHBrCH_3$, 2114-00-3; $PhC(OH)=C(CH_3)S(CH_3)_2^+Br^-$, 92126-07-3; $PhCOCH(CH_3)S(CH_3)_2^+$ Br^-, 19158-70-4; CH_3NCO, 624-83-9; $3,4\text{-}Cl_2C_6H_3COCHBrCH_3$, 87427-61-0; $ClCOCHBrCH_3$, 7148-74-5; $o\text{-}Cl_2C_6H_4$, 95-50-1; $3,4\text{-}Cl_2C_6H_3COCH(CH_3)S(CH_3)_2^+$ Br^-, 92126-10-8; $3,4\text{-}Cl_2C_6H_3NH_2$, 95-76-1; $3,4\text{-}Cl_2C_6H_3NHCH_2CO_2H$, 65051-17-4; $3,4\text{-}Cl_2C_6H_3N(NO)CH_2CO_2H$, 92126-15-3; $4\text{-}ClC_6H_4NH_2$, 106-47-8; $PhNH_2$, 62-53-3; $CH_3OC_6H_4NH_2$, 29191-52-4; $ClCH_2CO_2Et$, 105-39-5; $CH_3COCH_2CO_2Et$, 141-97-9; $PhCH_2Cl$, 100-44-7; $PhCS_2Na$, 3682-36-8; $CH_3CHBrCO_2H$, 598-72-1; $PhCS_2CH(CH_3)CO_2H$, 78751-36-7; PhN_3, 622-37-7; PhCHO, 100-52-7; PhCH=NOH, 932-90-1; PhC(Cl)=NOH, 698-16-8; $PhCONH_2$, 55-21-0; Cl_3CSH, 75-70-7; $EtO_2CCOCHClCO_2Et$, 34034-87-2; $(EtO_2C)_2$, 95-92-1; $PhCSNH_2$, 2227-79-4; PhCN, 100-47-0; $H_2NCH_2CO_2Et$·HCl, 623-33-6; $PhC(OEt)=NCH_2CO_2Et$, 15926-45-1; 5-phenyl-1,3,4-oxathiazol-2-one, 5852-49-3.

Synthesis of 5-Aryl-2*H*-tetrazoles, 5-Aryl-2*H*-tetrazole-2-acetic Acids, and [(4-Phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetic Acids as Possible Superoxide Scavengers and Antiinflammatory Agents[1]

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A series of 5-aryl-2*H*-tetrazoles, 5-aryl-2*H*-tetrazole-2-acetic acids, and [(4-phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetic acids were synthesized and tested in vitro for superoxide scavenging activity, in vivo in the carrageenan-induced rat paw edema assay, and in the reverse passive Arthus reaction. The hydroxy-substituted compounds were effective as in vitro scavengers of superoxide but were not effective as in vivo antiinflammatory agents.

A possible role of the superoxide anion radical (O_2^-) in the pathology of rheumatoid arthritis has been proposed.[2] Experimental evidence indicates that excess superoxide generates a chemotactic factor that perpetuates the inflammatory process.[3-6] Treatment of affected joints with polymer-stabilized bovine superoxide dismutase greatly lowers the activity of the inflammatory process and allows healing of the damaged tissue to ensue.[7] Stabilized superoxide dismutase was also found effective in both the reverse passive Arthus reaction and carrageenan-induced foot edema.[2] In lieu of isolating and modifying and enzyme from living tissue, a low molecular weight chemical agent was sought as a scavenger of superoxide and hence an antiinflammatory agent. Molecules containing gallic acid and catechol moieties are known to scavenge superoxide[8] and exhibit some antiinflammatory activity.[9] The selection of a proper carrier molecule into which these polyphenolic moieties could be incorporated is the subject of this paper.

(1) Taken from the Ph.D. Dissertation of J. R. Maxwell, University of North Dakota, 1983.
(2) McCord, J. M.; English, D. In "Enzymes as Drugs"; Holcenberg, J. S., Ed.; Wiley: New York, 1980; Chapter 12.
(3) Perez, H. D.; Weksler, B. B.; Goldstein, I. M. *Inflammation (N.Y.)* **1980**, *4*, 313–328.
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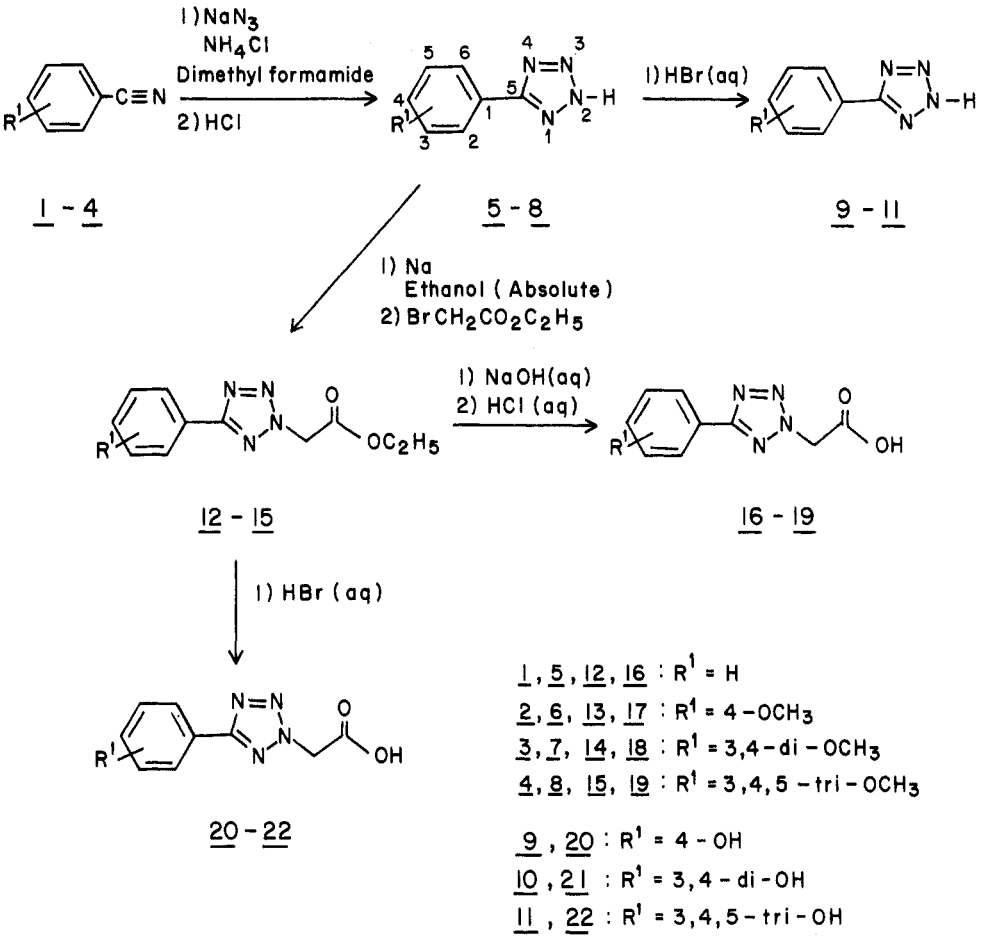
0022-2623/84/1827-1565$01.50/0

Scheme I



Substituted tetrazoles, such as 5-phenyl-2*H*-tetrazole-2-propionic acid, have been demonstrated to possess antiinflammatory activity.[10] Certain triazole-containing molecules including [[4-phenyl-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazol-3-yl]thio]acetic acid hydrazide, also have been shown to have antiinflammatory activity.[11] Using these compounds as models, a series of 5-aryl-2-*H*-tetrazoles (Scheme I), 5-aryl-2*H*-tetrazole-2-acetic acids (Scheme I), and [(4-phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)-thio]acetic acids (Scheme II) were synthesized. These particular compounds were chosen for their synthetic simplicity and stability to aryl ether cleavage conditions. The basic carrier molecules possess little, if any, antiinflammatory activity. The in vitro superoxide scavenging and in vivo antiinflammatory activities were investigated.

Chemistry. Various 5-aryl-2*H*-tetrazoles (**5–8**) were obtained by the reaction of the appropriate methoxy-substituted benzonitrile (**1–4**) with NaN_3.[12] These compounds (**5–8**) were further treated with aqueous HBr to cleave the methyl ethers and form the hydroxy-substituted tetrazoles (**9–11**).[13] The methoxy-substituted tetrazoles (**5–8**) were also treated with ethyl bromoacetate in the presence of sodium ethoxide to form ethyl 5-aryl-2*H*-tetrazole-2-acetates (**12–15**).[12] The methyl aryl ethers and ethyl ester portions of these molecules were cleaved in the presence of aqueous HBr (**20–22**).[13] Compounds **12–15** were hydrolyzed with NaOH to cleave only the ethyl esters (**10–19**).

Various benzoic acid 2-[(phenylamino)thioxomethyl]-hydrazides (**35–38**) were formed by the reaction of the appropriate benzoic acid hydrazide (**31–34**) with phenyl isothiocyanate.[11] Compounds **35–38** were cyclized into various 4-phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiols (**39–42**) with aqueous NaOH.[11] The thiols **39–42** were further treated with ethyl bromoacetate to form ethyl [(4-phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetates (**43–46**). The methyl aryl ethers and ethyl esters were cleaved with aqueous HBr to form the hydroxy-substituted [(4-phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetic acids (**51–53**).[12] The ethyl ester was hydrolyzed with NaOH to produce compounds **47–50**.

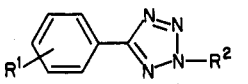
Results and Discussion

The in vitro superoxide scavenging activity of the 5-aryl-2*H*-tetrazoles (Table I) increases as the number of free phenolic groups increase. The methoxy compounds **6–8** as well as the unsubstituted (**5**) and monohydroxy (**9**) derivatives show no activity. The introduction of an acetic acid side chain into the tetrazoles **16–22** causes an increase in the scavenging activity of the trihydroxy compound **22** and a decrease in the activity of the corresponding dihydroxy compound **21**. The more active compounds **10, 11, 21, 22** were tested at three different concentrations to check for any biphasic responses; there were none.

The scavenging activity of the triazoles **47–53** also increased with an increased number of free phenolic moieties (Table II). Unlike the corresponding tetrazole, the (monohydroxyaryl)triazole derivative **51** exhibited scavenging

(10) Singh, H.; Chawla, A. S.; Kapoor, U. K.; Paul, D.; Malhotra, R. K. *Prog. Med. Chem.* **1980**, *17*, 151–183.
(11) Jaiswal, R. K.; Parmar, S. S.; Singh, S. P.; Barthwal, J. P. *J. Heterocycl. Chem.* **1979**, *16*, 561–565.
(12) Raman, K.; Parmar, S. S.; Singh, S. P. *J. Heterocycl. Chem.* **1980**, *17*, 1137–1139.
(13) Haslam, E. In "Protective Groups in Organic Chemistry"; McOmie, J. F. W., Ed.; Plenum Press: New York, 1973; Chapter 4 and references therein.

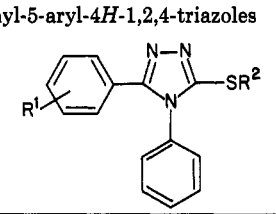
Table I. Chemical and Physical Data for the 5-Aryl-2*H*-tetrazoles



no.	R^1	R^2	mp, °C (lit.)	yield, %	formula[b,c]	SOD scavenging act., % inhib of control[d]	concn, × 10^4 M
5	H	H	213–215 (213–215)[16]	89	$C_7H_6N_4$	0.0 ± 0.0	1.0
6	4-OCH_3	H	227–229 (228)[17]	78	$C_8H_8N_4O$	0.0 ± 0.0	1.0
7	3,4-$(OCH_3)_2$	H	206–207	78	$C_9H_{10}N_4O_2$	0.0 ± 0.0	1.0
8	3,4,5-$(OCH_3)_3$	H	201–202 (199–200)[18]	89	$C_{10}H_{12}N_4O_3$	0.0 ± 0.0	1.0
9	4-OH	H	237–238[a]	76	$C_7H_6N_4O$	0.0 ± 0.0	2.0
10	3,4-$(OH)_2$	H	256–257[a]	79	$C_7H_6N_4O_2$	37.5 ± 1.8	1.0
						23.0 ± 1.8	0.5
						6.9 ± 1.1	0.1
11	3,4,5-$(OH)_3$	H	282–283[a]	72	$C_7H_6N_4O_3 \cdot {}^3/_4H_2O$	66.9 ± 0.0	1.0
						48.4 ± 3.5	0.5
						16.0 ± 0.9	0.1
12	H	$CH_2CO_2C_2H_5$	78–79 (78–79)[17]	63	$C_{11}H_{12}N_4O_2$		
13	4-OCH_3	$CH_2CO_2CH_2H_5$	98–99 (99–99.5)[17]	67	$C_{12}H_{14}N_4O_3$		
14	3,4-$(OCH_3)_2$	$CH_2CO_2CH_2H_5$	104–106	59	$C_{13}H_{16}N_4O_4$		
15	3,4,5-$(OCH_3)_3$	$CH_2CO_2C_2H_5$	103–104	52	$C_{14}H_{18}N_4O_5$		
16	H	CH_2CO_2H	180–181 (182–184)[19]	75	$C_9H_8N_4O_2$	0.0 ± 0.0	1.0
17	4-OCH_3	CH_2CO_6H	172–173	67	$C_{10}H_{10}N_4O_3$	0.0 ± 0.0	1.0
18	3,4-$(OCH_3)_2$	CH_2CO_2H	154–155	73	$C_{11}H_{12}N_4O_4$	0.0 ± 0.0	1.0
19	3,4,5-$(OCH_3)_3$	CH_2CO_2H	192–193	76	$C_{12}H_{14}N_4O_5$	0.0 ± 0.0	1.0
20	4-OH	CH_2CO_2H	194–195[a]	63	$C_9H_8N_4O_3$	0.0 ± 0.0	1.0
21	3,4-$(oH)_2$	CH_2CO_2H	220–221[a]	70	$C_9H_8N_4O_4$	18.8 ± 3.1	1.0
						7.9 ± 2.1	0.5
						0.0 ± 0.0	0.1
22	3,4,5-$(OH)_3$	CH_2CO_2H	242–243[a]	62	$C_9H_8N_4O_5$	78.0 ± 4.7	1.0
						55.9 ± 2.4	0.5
						15.3 ± 2.7	0.1

[a] Decomposed. [b] Analysis: C, H, N. [c] NMR and IR spectral data consistent with assigned structures. [d] 95% confidence interval.

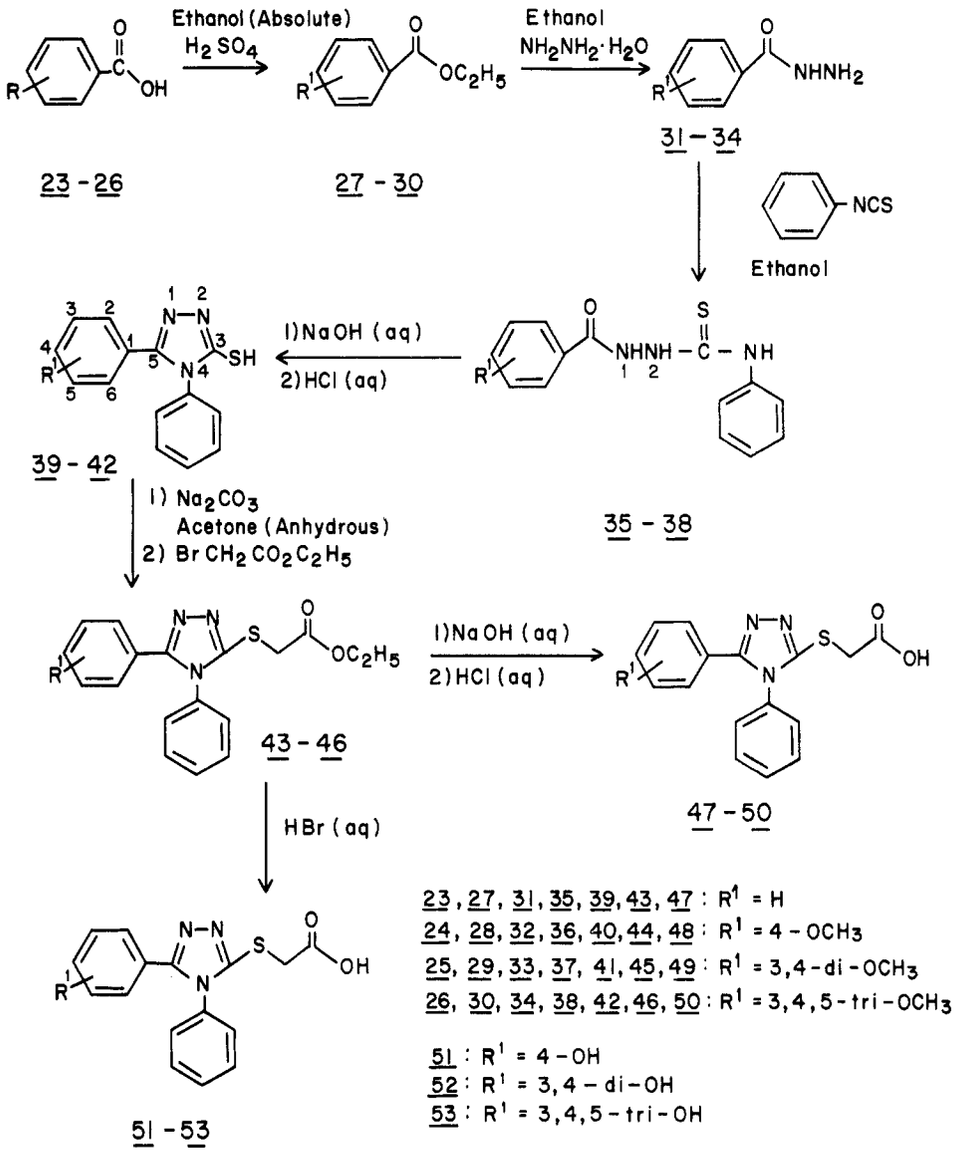
Table II. Chemical and Physical Data for the 4-Phenyl-5-aryl-4*H*-1,2,4-triazoles



no.	R^1	R^2	mp, °C (lit.)	yield, %	formula[b,c]	SOD scavenging act., % inhib of control[d]	concn, × 10^4 M
31	H	H	280–283[a]	91	$C_{14}H_{11}N_3S$		
40	4-OCH_3	H	284–285[a]	98	$C_{15}H_{13}N_3OS$		
41	3,4-$(OCH_3)_2$	H	234–235	99	$C_{16}H_{15}N_3O_2S$		
42	3,4,5-$(OCH_3)_3$	H	210–211 (210–212)[11]	93	$C_{17}H_{17}N_3O_3S$		
43	H	$CH_2CO_2C_2H_5$	101–102	91	$C_{18}H_{17}N_3O_2S$		
44	4-OCH_3	$CH_2CO_2C_2H_5$	166–168	72	$C_{19}H_{19}N_3O_3S$		
45	3,4-$(OCH_3)_2$	$CH_2CO_2C_2H_5$	122–124	88	$C_{20}H_{21}N_3O_3S$		
46	3,4,5-$(OCH_3)_3$	$CH_2CO_2C_2H_5$	137–138 (137–178)[11]	79	$C_{21}H_{23}N_3O_5S$		
47	H	CH_2CO_2H	249–251[a]	98	$C_{16}H_{15}N_3O_2S$	0.0 ± 0.0	1.0
48	4-OCH_3	CH_2CO_2H	230–233	92	$C_{17}H_{17}N_3O_3S$	0.0 ± 0.0	1.0
49	3,4-$(OCH_3)_2$	CH_2CO_2H	216–218	95	$C_{18}H_{19}N_3O_4S$	0.0 ± 0.0	1.0
50	3,4,5-$(OCH_3)_3$	CH_2CO_2H	202–204	96	$C_{19}H_{21}N_3O_5S$	0.0 ± 0.0	1.0
51	4-OH	CH_2CO_2H	254–255[a]	92	$C_{16}H_{15}N_3O_3S$	11.6 ± 4.8	1.0
52	3,4-$(OH)_2$	CH_2CO_2H	248–251[a]	94	$C_{16}H_{15}N_3O_4S \cdot {}^1/_2H_2O$	21.5 ± 1.1	1.0
						11.8 ± 2.5	0.5
						8.3 ± 5.0	0.1
53	3,4,5-$(OH)_3$	CH_2CO_2H	245–248[a]	95	$C_{16}H_{15}N_3O_5S$	65.7 ± 6.5	1.0
						22.1 ± 4.1	0.5
						16.7 ± 1.8	0.1

[a] Decomposed. [b] Analysis: C, H, N. [c] NMR and IR spectral data consistent with assigned structures. [d] 95% confidence interval.

Scheme II



activity. The scavenging activities of compounds **52** and **53** were similar to those of compounds **10** and **11** (Table I).

According to the results of the in vitro testing (Tables I and II), compounds **11**, **22**, and **53** were the most active and were tested further for in vivo antiinflammatory activity. Table III gives the data for the carrageenan-induced rat paw edema assay. Compounds **11**, **22**, and **53** were not significantly active compared to phenylbutazone and dexamethasone. In the reverse passive Arthus reaction, compound **11** was not significantly active, whereas **22** and **53** were actually proinflammatory.

An explanation for the lack of in vivo antiinflammatory activity of **11**, **22**, and **53** may be related to the similarity of their structure to the endogenous catechols. In vivo pathways for the metabolism of polyphenols are efficient and rapid.[14] Since the compounds were administered orally, they may not have reached the site of inflammation before being excreted or metabolized. This difficulty may possibly be overcome by hindering the hydroxyl groups so the metabolizing enzymes cannot transform them.

Table III. Pharmacological Data for the 5-Aryl-2*H*-tetrazoles and the 4-Phenyl-5-aryl-4*H*-1,2,4-triazoles

	carrageenan		reverse passive Arthus reaction[b,c]	
compd	concn, mg/kg	edema,[a] % inhib	concn, mg/kg	mean index	% inhib
11	32	6	30	42.6	−5.0
22	32	14	30	52.0	−28.7
53	32	18	30	52.0	−28.2
phenylbutazone	32	47			
dexamethasone	0.3	69			

[a] Six Charles River rats were used for each determination; test compounds were dissolved in a solution of 1:1 *N*-methyl-D-glucamine and water. [b] Five Charles River rats were used for each determination; test compounds were dissolved in a solution of 1:1 *N*-methyl-D-glucamine and water. [c] Control: mean index, 40.4; percent inhibition, 0.0.

Moore and Swingle have recently reported the testing of such a hindered phenol.[20] 2,6-Di-*tert*-butyl-4-(2-the-

(14) Bowman, W. C.; Rand, M. J. In "Textbook of Pharmacology", 2nd ed.; Blackwell Scientific Publications: Oxford, 1980; Chapter 40.

(15) Pearl, I. A.; Beyer, D. L. *J. Am. Chem. Soc.* **1955**, *77*, 3660–3662.

noyl)phenol exhibited significant antioxidant and antiinflammatory activities. The compound was not tested for superoxide scavenging activity, so it is not known if the antiinflammatory activity is related to superoxide scavenging.

Experimental Section

Chemistry. All new compounds were analyzed for carbon, hydrogen, and nitrogen by Guelph Chemical Laboratories, Ltd. (Guelph, Ontario, Canada) or MicAnal (Tuscon, AZ) and agreed to ±0.4% of the calculated values. Melting points were taken in open capillary tubes with a Mel-Temp capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet MX-S spectrometer as Nujol mulls. NMR spectra were obtained on a 90-MHz Varian EM-390 instrument using tetramethylsilane as an internal reference and Me_2SO-d_6 as solvent. All IR and NMR spectral data were in agreement with the assigned structures. The carrageenan-induced rat paw edema assay and the reverse passive Arthus reaction were performed under the direction of Dr. Ivan Otterness at Pfizer Pharmaceuticals, Groton, CT.

General Procedure for 5-Aryl-2*H*-tetrazoles 5–8.[12] A mixture of the appropriate benzonitrile (**1–4**) (0.2 mol), NaN_3 (0.22 mol), and NH_4Cl (0.22 mol) in 200 mL of distilled dimethylformamide (DMF) was heated under reflux for 17 h with stirring. The hot solution was poured over crushed ice and acidified to pH 2 with 2 N HCl. The solid that separated was filtered, washed with ice-cold water, and recrystallized from 95% ethanol.

General Procedure for Ethyl 5-Aryl-2*H*-tetrazole-2-acetates 12–15.[12] The appropriate 5-aryl-2*H*-tetrazole (**5–8**) (0.1 mol) was dissolved in a solution prepared by reacting Na (0.1 mol) with 200 mL of absolute ethanol. The solution was refluxed with stirring and ethyl bromoacetate (0.1 mol) was added in three portions over a period of 0.5 h. After heating under reflux for 16 h, the reaction mixture was filtered while hot to remove precipitated sodium bromide; the solvent was removed on a rotary vacuum evaporator. The crude product was recrystallized from 95% ethanol.

General Procedure for Ethyl Benzoates 27–30. A mixture of the appropriate benzoic acid (**23–26**) (0.3 mol) and 6 mL of concentrated H_2SO_4 in 300 mL of anhydrous ethanol was heated under reflux for 20 h. The reaction mixture was concentrated on a rotary vacuum evaporator, extracted with ether, and washed with saturated $NaHCO_3$. The ether layer was dried ($MgSO_4$), and the ether was removed on a rotary vacuum evaporator. If the resulting ester was a solid, it was recrystallized from 95% ethanol; if liquid, it was distilled under reduced pressure.

General Procedure for Benzoic Acid Hydrazides 31–34.[11,15] A mixture of the appropriate ethyl benzoate (**27–30**) (0.1 mol), hydrazine hydrate (0.25 mol), and 30 mL of 95% ethanol was heated under reflux for 6 h. The solvent was removed on a rotary vacuum evaporator and the residue was poured into 200 mL of cold water. The solid that formed was collected, washed with ice-cold water, and recrystallized from 95% ethanol. (In the preparation of **33**, no solvent was used.[15])

General Procedure for Benzoic Acid 2-[(Phenylamino)-thioxomethyl]hydrazides 35–38.[11] Equimolar quantities of the appropriate benzoic acid hydrazide (**31–34**) (0.1 mol) and phenyl isothiocyanate (0.1 mol) in 125 mL of absolute ethanol were heated under reflux for 4 h. The solvent was removed on a rotary vacuum evaporator. The resulting solid was filtered, dried, and recrystallized from 95% ethanol.

General Procedure for 4-Phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiols 39–42.[11] A mixture of the appropriate benzoic acid 2-[(phenylamino)thioxomethyl]hydrazide (**35–38**) (0.01 mol) and 100 mL of 2 N NaOH was heated under reflux for 3 h. The solution was cooled and acidified to pH 2 with concentrated HCl. The solid that precipitated was filtered, washed with water, and recrystallized from 95% ethanol.

General Procedure for Ethyl [(4-Phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetates 43–46.[11] A mixture of ethyl bromoacetate (0.015 mol), the appropriate 4-phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiol (**39–42**) (0.015 mol), and anhydrous K_2CO_3 (0.018 mol) in 60 mL of anhydrous acetone was heated under reflux for 8 h. The reaction mixture was filtered to remove precipitated KBr and the solvent was removed on a rotary vacuum evaporator. The crude solid was recrystallized from 95% ethanol.

General Procedure for Methyl Aryl Ether Cleavage (9–11, 20–22, 51–53).[13] A mixture of the appropriate methyl aryl ether (**12–15, 43–46**) (500 mg) and 5 mL of distilled, 48% aqueous HBr was heated under reflux in an oil bath (135–140 °C) for 4 h under a nitrogen atmosphere. The reaction mixture was cooled below 0 °C for 12 h; the resulting solid was filtered, washed with ice-cold water, and recrystallized from hot water and/or 95% ethanol. Ethyl esters, if present, were also cleaved by this procedure.

General Procedure for Ethyl Ester Cleavage (16–19, 47–50). A mixture of the appropriate ethyl ester (**12–15, 43–46**) (500 mg) and 5 mL of 2 N NaOH was heated under reflux for 4 h. The hot reaction mixture was acidified to pH 2 with concentrated HCl. The resulting solid was recrystallized from 95% ethanol and/or hot water. This procedure left the methyl aryl ethers intact.

Superoxide Scavenging Activity Assay. The scavenging activities of the compounds of interest were assayed by the method of Fried.[21] The phosphate buffer (0.1 M, pH 7.6) used in this assay was prepared according to the following description. Solution A contained 22.6 g of $NaH_2PO_4 \cdot H_2O$, 262 mg of 1,1,4,7,7-diethylenetriaminepentaacetic acid (DTPA), and enough water to make 1.0 L. Solution B contained 71.7 g of $Na_2HPO_4 \cdot 12H_2O$, 262 mg of DTPA, and enough water to make 1.0 L. The final buffer solution was prepared by mixing 8.5 mL of solution A and 91.5 mL of solution B and diluting to 200 mL. Gelatin (76 mg) was dissolved in this solution before use by gentle heating on a steam bath. This gave a final gelatin concentration of 1 mg/2.6 mL. Each assay tube (13 × 100 mm) contained 100 μL of a dimethyl sulfoxide (Me_2SO) solution of the compound to be tested, 100 μL of a nitro blue tetrazolium (NBT) solution (4 mg/mL in Me_2SO), 100 μL of a phenazine methosulfate (PMS) solution (10 mg/100 mL in phosphate buffer without added gelatin), and 2.6 mL of phosphate buffer. The reaction was initiated by the addition of 100 μL of the reduced form of β-nicotinamide-adenine dinucleotide (NADH) (1.5 mM in phosphate buffer without added gelatin) solution. This gave a final volume of 3.0 mL. Care was taken to perform any manipulations of NBT and PMS in low light and to keep the NADH solution on ice. Immediately before and after the addition of the NADH solution, each assay tube was thoroughly mixed on a vortex mixer. After standing in the dark for 10 min, the contents of the assay tubes were transferred to a square 10-cm glass cuvette, and the absorbance was measured at 540 nm with a Beckman Model 25 spectrometer. All readings were taken against a blank containing everything but the NADH solution. The scavenging activity of the compounds tested were examined statistically by the "analysis of variance" method.[22]

Pharmacology. Carrageenan-Induced Rat Paw Edema Assay. This assay was performed according to the method of Lombardino et al.[23] Compounds **11**, **22**, and **53** were dissolved in a 1:1 solution of *N*-methyl-D-glucamine and water; control animals received vehicle only. One hour after oral administration of the test compound (32 mg/kg of body weight), the initial volume (V_I) of the rat hind paw was measured and 0.05 mL of a 1% suspension of carrageenan in 0.9% saline was injected into the subplantar area of the right hand paw. Exactly 3 h later, the volume (V_F) of this paw was measured again. Percent inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = [1 - [(V_F \text{ compound} - V_I \text{ compound})/(V_F \text{ control} - V_I \text{ control})]] \times 100$$

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Six Charles River rats were used for each determination.

Reverse Passive Arthus Reaction. This assay was performed according to the procedure of Chang and Otterness.[24] The animals used were injected intravenously through the tail vein with 1.0 mL of 0.9% saline containing 2.5 mg of Evans Blue and 5.0 mg of ovalbumin, followed immediately by intracutaneous injection of 0.03 mL of rabbit antiovalbumin antiserum diluted with sufficient 0.9% saline to contain 3.65 mg of antibody/mL. The compound to be tested (**11**, **22**, or **53**) (30 mg/kg of body weight) was administered orally in a 1:1 *N*-methyl-D-glucamine and water solution 1 h before the injection of the ovalbumin. The mean index of the reaction was calculated 3-h postinjection as the product of the diameter of the reaction site (indicated by the accumulation of Evans Blue) and the intensity score of the reaction (a subjective score of 1–4). Five Charles River rats were used for each determination.

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Supplementary Material Available: Tables containing complete spectral and physical data for all compounds synthesized (19 pages). Ordering information is given on any current masthead page.

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Probes for Narcotic Receptor Mediated Phenomena. 7.[1] Synthesis and Pharmacological Properties of Irreversible Ligands Specific for μ or δ Opiate Receptors

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Syntheses of affinity reagents for opiate receptors based on the fentanyl, *endo*-ethenotetrahydrooripavine, and etonitazene carbon–nitrogen skeletons are described. The isothiocyanate, bromoacetamido, and methylfumaramido alkylating functions were employed in these compounds, some of which had previously been shown to be μ specific (**12**, BIT) and δ specific (**8**, FIT and **19**, FAO) in vitro. Antinociceptive activity of the title compounds was determined in the mouse hot-plate test, which revealed that certain compounds in each class showed morphine-like activity. The binding EC_{50} values against [^{3}H]Dalamid for opiate receptors in NG108-15 (δ receptors) and rat brain membranes (μ + δ receptors) are also reported. With this type of experiment, it was possible to independently measure the apparent affinity of the etonitazene congeners **12**–**14** for the μ and δ receptors.

The concept of multiplicity of opiate receptors, originally proposed by Martin[2] to account for differing pharmacological effects of several opiates in the spinal dog, has received much support from binding and other pharmacological studies.[3-8] The three major types of opioid receptors in the CNS are commonly referred to as μ, δ, κ.[9] Some opioids display considerable selectivity in their interaction with these receptor types. The endogenous opioid peptides enkephalin and dynorphin are relatively selective ligands for δ and κ receptors, respectively.[9,10] Important questions raised by these observations are whether the receptors are structurally related, and what are the physiological roles of these receptors. Answers to these questions can come from experiments performed with opioids which bind irreversibly to one or another of the receptor types to the exclusion of the others. Many attempts at the preparation of affinity reagents for opioid receptors have been made;[11-22] some of those based on the 3,14-dihydroxy-4,5-epoxymorphinan system are useful probes for the μ receptor.[16,17]

With the goal of obtaining opioids specific for opiate receptor subpopulations, we have prepared three classes

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