Alkylating Prazosin Analogue: Irreversible Label for α_1-Adrenoceptors

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A series of prazosin analogues comprised of *N*-acyl derivatives of *N'*-(4-amino-6,7-dimethoxyquinazolin-2-yl)piperazine was prepared and the nature of their binding to α_1-adrenoceptors was investigated. Derivatives with α,β-unsaturated acyclic acyls had some affinity but no irreversible action at the receptor. Other potent compounds, also without irreversible activity, contained cinnamoyl or (phenylamino)thiocarbonyl residues. High affinity and irreversible binding were obtained with a bicyclo[2.2.2]octa-2,5-dien-2-ylcarbonyl derivative. The conjugated double bond in this compound was in about the same position and distance from the pharmacophore as in some of the above compounds of high affinity but with no irreversible action. Two consecutive recognition steps were thought to be involved in irreversible blockade: reversible binding of the pharmacophore part of the molecule to the binding site of the receptor, followed by reaction of the chemoreactive part with an adjacent nucleophile of the receptor. The present results suggest that for the second step to occur efficiently, some affinity for the receptor must be present even in the chemoreactive part of the molecule; simple spanning of the binding and nucleophile sites of the receptor was insufficient.

Since the introduction of compounds with high affinity and receptor specificity, chemoreactive labels for hormonal receptors have become important research tools in pharmacology. In the field of β-adrenoceptors, suitable alkylating β-blockers were used[1,2] and an alkylating agonist was also prepared.[3] For analogous studies of α_1-adrenoceptors, phenoxybenzamine, *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ), and tetramine disulfides were used; however, these have a number of disadvantages. Phenoxybenzamine was not very specific;[4] in addition to α_1-adrenoceptors, it interacted with α_2-adrenoceptors,[5] histamine receptors,[6] dopamine receptors,[7] and muscarinic receptors.[8] The receptor specificity of *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline was also low.[9-12] In addition, this compound was used at concentrations about 10^4 higher than those of the more specific chemoreactive labels. The tetramine disulfide pyrextramine was used to selectively block peripheral α_1-adrenergic responses.[13] However, more recently, pyrextramine was shown to block peripheral α_2-adrenergic responses as well,[14] which suggested a lack of specificity for α_1-adrenergic responses. On the other hand, photoreactive labels for α_1-adrenoceptors of high affinity and specificity were prepared.[15-17] These probes were analogues of prazosin (compound **1**, Table I).

In this work we evaluated a number of prazosin analogues for potential use as chemoreactive labels. A somewhat broader structure–activity study had to be made to find structures suitable for further substitutions. In the study several compounds with comparable affinity and one with considerably higher affinity to the receptor than that of the parent drug were found, and eventually the desired irreversible label was made.

Chemistry

Prazosin analogues were prepared by acylation of 4-amino-6,7-dimethoxy-2-(piperazin-1-yl)quinazoline with acyl chlorides, isocyanates, or isothiocyanates; the structures of the compounds are in Table I. The required piperazine derivative was an intermediate in the originally used synthesis of prazosin[18] and was obtained either by a modified method[17] or by acid hydrolysis of prazosin. Some of the compounds synthesized were described previously, but were reprepared to obtain pertinent pharmacological data; for new compounds compare Table II.

Pharmacological Results

The ability of the compounds to compete with [^{3}H]-prazosin for binding sites on rat cerebral cortical membranes was measured, and IC_{50} values were calculated from the displacement curves. Preincubation of membranes with compounds selected from the series, followed by extensive washing and then assaying for saturation of specific [^{3}H]prazosin binding established if these compounds were irreversibly bound to receptor sites. To prevent artifacts in the establishment of irreversibility, the receptors remaining after this treatment were characterized by both their concentrations (B_{max}) and affinities (K_D) from Scatchard plots of saturation data. An irreversible probe was expected to affect only the former, not the latter. This evaluation was not performed on compounds with affinities too low to be of practical use as receptor probes.

The results are collected in Table I. In compounds **2** and **3** the furan nucleus of prazosin (**1**) was replaced by a chemoreactive α,β-unsaturated acyl group which may potentially react with a sulfhydryl group of the receptor. Both of these compounds had an affinity for α_1-adrenoceptor, but did not block it irreversibly.

The cinnamoyl analogue of prazosin, **4**, was previously shown to be able to reduce blood pressure in conscious, spontaneously hypertensive rats.[19] Evaluated by the

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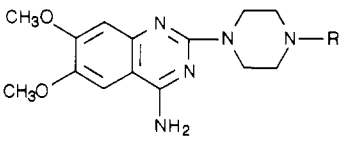
Table I. Effects of Analogues on α_1-Adrenoceptor Binding of [^{3}H]Prazosin



no.	R	−log IC_{50}[a]	irreversibility[b] (% decrease in B_{max})	concn,[c] nM
1	CO-2-furyl, prazosin[d]	9.79 ± 0.15	0	100
2	COCH=CHCH=CHCOOCH$_3$ (trans,trans)	7.41 ± 0.05	0	1000
3	COCH=CHCH=CHCH$_3$ (trans,trans)[d]	8.36 ± 0.06	0	100
4	COCH=CHC$_6$H$_5$ (trans)[d]	9.16 ± 0.33	0, K_D effect	100
5	COCH=CH-*p*-C$_6$H$_4$F (trans)	7.64 ± 0.18	3 ± 9	100
6	COCH=CH-*p*-C$_6$H$_4$Cl[d]	7.90	NT[e]	
7	COCH=CH-*p*-C$_6$H$_4$Br[d]	7.10	NT	
8	COCH=CH-*m*-C$_6$H$_4$F	7.68	NT	
9	COCH=CH-*p*-C$_6$H$_4$OCH$_3$ (trans)[d]	8.47 ± 0.43	0	100
10	COCH=CH-*p*-C$_6$H$_4$-NCS	6.99	1%	100
11	COCH=CH-*p*-C$_6$H$_4$NHCOCH$_2$Br (trans)	7.17 ± 0.14	0, K_D effect	100
12	COCH=CH-*p*-C$_6$H$_4$NHCOCH=CHCOOCH$_3$ (trans, trans)	7.53 ± 0.06	0	100
13	COCF=CHC$_6$H$_5$	7.40 ± 0.10	0	100
14	COCH$_2$CH$_2$C$_6$H$_5$[d]	8.47	NT[e]	
15	COCH$_2$OC$_6$H$_5$[d]	7.90	NT	
16	COCH=CHCOC$_6$H$_5$ (trans)	7.06	NT	
17	CO-2-naphthyl	7.31	NT	
18	COCHCH$_2$CHC$_6$H$_5$ (trans)[d]	9.29	NT	
19	COCHOCHC$_6$H$_5$ (trans)	7.88 ± 0.21	0	100
20	CSNHC$_6$H$_5$[d]	11.16	NT	
21	CSNHC$_6$H$_{11}$	8.27	NT	
22	CSNH-*p*-C$_6$H$_4$N$_3$	7.70	NT	
23	CSNH-*p*-C$_6$H$_4$NHCSNCH$_2$CH$_2$	7.01	NT	
24	CONHC$_6$H$_5$[d]	7.56	NT	
25	CO-*m*-C$_6$H$_4$CH$_2$Cl	7.93	NT	
26	COC$_6$H$_3$-*m*-I-*p*-NCS	7.29	NT	
27	COCH$_2$CH$_2$CH$_2$CH$_2$-*p*-C$_6$H$_4$NHCOCH=CHCOOCH$_3$ (trans)	8.35 ± 0.14	0	100
28	SO$_2$-*p*-C$_6$H$_4$CH$_3$[d]	7.94	NT	
29	SO$_2$-2-thienyl	7.47	NT	
30	CO-bicyclo[2.2.1]hept-2-en-5-yl[d]	8.80 ± 0.22	4 ± 6	10
31	CO-bicyclo[2.2.2]octa-2,5-dien-2-yl	8.12 ± 0.22	54 ± 3.7	10

[a] Results are the averages of between three and five separate experiments ± 1 SEM or of duplicate experiments when SEM is not given. [b] Results are the averages of at least three separate experiments. The control B_{max} value was 267 ± 21 fmol/mg protein (n = 11) and the K_D for [^{3}H]prazosin was 0.412 ± 0.061 nM. [c] The concentration used to test irreversibility of the compound. [d] Previously described compounds: **1**;[18] **3**;[28] **14**;[28] **15**;[28] **4**;[19] **7**;[19] **9**;[19] **18**;[28,29] **20**;[30] **24**;[30] **28**;[31] **30**.[32] [e] NT = not tested.

present methods, compound **4** had good affinity but no irreversible action in spite of its reactive carbonyl conjugated double bond. Ring substitution in the cinnamoyl analogue by nonreactive groups, compounds **5–9**, resulted only in a decrease in affinity, whereas no irreversibility was gained in this manner. Compounds **10–12** contained a cinnamoyl residue substituted with reactive groups, but there was no irreversible binding to the receptor either. Substitution by fluorine on the double bond (**13**) also only decreased affinity without gaining irreversibility. To widen the field of search, compounds **14–18** were investigated, which were somewhat related to the cinnamoyl analogue but did not necessarily have reactive groups. When the double bond of the cinnamoyl residue was replaced by a single bond (**14**, **15**), affinity decreased considerably. Insertion of a carbonyl between the double bond and the benzene nucleus of the cinnamoyl moiety, compound **16**, also decreased the affinity. When the double bond of the cinnamoyl residue was conserved as a part of an additional cyclic structure (naphthalene derivative, **17**), the affinity was also decreased considerably. A cyclopropane moiety might be considered a homologue of a double bond and this analogy was applicable here; the cyclopropane derivative (**18**) had affinity similar to that of the cinnamoyl compound **4**. Consequently, the structural variation of **18** was performed to gain a chemically reactive compound. Ethylene oxide had obvious similarity to cyclopropane and the chemoreactivity required of irreversible labels. Unfortunately, when an oxirane analogue (compound **19**) was made, its affinity for receptors was 2 orders of magnitude lower than that of cyclopropane and it lacked irreversible action.

A phenylthiourea analogue of prazosin, compound **20**, was found to have much higher affinity for α_1-adrenoceptors than prazosin, unfortunately all further variations of its structure yielded only compounds of low affinity. Replacement of the sulfur in **20** by oxygen to give the urea (**24**) considerably decreased the affinity; saturation of the phenyl ring to cyclohexyl (**21**) also led to a decrease. Substitutions of the phenylthiourea residue by photo- or chemoreactive groups, compounds **22** and **23**, respectively, also decreased the affinity.

Compounds **25–27** contained acyl groups with a benzene nucleus. Compound **26** closely resembled the high-affinity photolabel for α_1-adrenoceptors,[16] the only change being that the azido group of the photolabel was replaced by isothiocyanate. Electronic and steric parameters of these groups are quite similar;[20] nevertheless, isothiocyanate **26**

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Table II. Prazosin Analogues

no.	yield, %	mp, °C	recryst solvent[a]	chrom solvent[b]	R_f	formula
2	36	230–232	A			$C_{21}H_{25}N_5O_5$
5	65	184	B			$C_{23}H_{24}N_5O_3F\cdot 3H_2O$
8	75	178	G			$C_{23}H_{24}N_5O_3F\cdot 3H_2O$[c]
10	37	195–200	E	I	0.42	$C_{24}H_{24}N_6O_3S$
11	42	250 dec	A			$C_{25}H_{27}N_6O_4Br\cdot H_2O$
12	43	297 dec	D			$C_{28}H_{30}N_6O_6$
13	21	235	B	I	0.57	$C_{23}H_{24}N_5O_3F\cdot HCl\cdot 0.5H_2O$[c]
16	54	226–229	D			$C_{24}H_{25}N_5O_4\cdot H_2O$
17	57	286	C	II	0.34	$C_{25}H_{25}N_5O_3\cdot HCl\cdot 0.5CH_2Cl_2$
19	54	117–119	A	I	0.65	$C_{23}H_{25}N_5O_4\cdot H_2O$
21	67	amorphous		I	0.59	$C_{21}H_{30}N_6O_2S$
22	97	172	D			$C_{21}H_{23}N_9O_2S$
23	50	187–190		III	0.40	$C_{24}H_{28}N_8O_2S_2$
25	51	230 dec	D			$C_{22}H_{24}N_5O_3Cl$
26	40	218–225 dec	F			$C_{22}H_{21}N_6O_3SI$
27	55	245–248	A			$C_{30}H_{36}N_6O_6$
29	84	amorphous		I	0.61	$C_{18}H_{21}N_5O_4S_2$
31	85	265–267	G	I	0.44	$C_{23}H_{27}N_5O_3\cdot 0.5CH_3OH$

[a] A = $CH_3CO_2C_2H_5$; B = aqueous C_2H_5OH; C = $CH_3CO_2C_2H_5$-CH_2Cl_2; D = $CH_3CO_2C_2H_5$-CH_3OH; E = C_2H_5OH–petroleum ether; F = $CH_3CO_2C_2H_5$–petroleum ether; G = $CH_3CO_2C_2H_5$–CH_3OH–C_6H_{12}. [b] I = CH_2Cl_2–CH_3OH, 9:1; II = $CHCl_3$–CH_3OH, 92.5:7.5; III = benzene–CH_3OH, 4:1. [c] Deviations in analysis larger than 0.4%. (**8**) C: calcd, 56.20, found, 55.72; N: calcd, 14.15, found, 13.66. (**13**) N: calcd, 14.49, found, 13.99.

had a much lower affinity and thus was unsuitable for the present purpose. We had a similar experience with some β-adrenergic ligands (unpublished material) and suspect that differences in hydration of these two groups might have been the cause of the reduced affinity. Compound **27** was similar to a successful photochemical probe for α_1-adrenoceptors,[15] but did not have irreversible action. Compounds **28** and **29** were sulfonamides and had only low affinity.

Ultimately, acyls containing bicyclic hydrocarbons were used to replace the furoyl residue of prazosin. Physiological effects of two compounds of this type, one derived from adamantanecarboxylic acid and another from bicyclo[2.2.2]oct-2-ene-5-carboxylic acid (unspecified stereochemistry), were evaluated previously.[21] The present results showed that compounds of high affinity were obtained with similar bicyclic hydrocarbon moieties, e.g. compound **30**. Finally a prazosin derivative containing bicyclo[2.2.2]octa-2,5-diene-2-carboxylic acid was synthesized (**31**). Compound **31** had a carbonyl conjugated double bond which could covalently add, for example, the mercapto group of cysteine residues; no such double bond was in compound **30**.

Compound **31** was then found to bind to the receptors irreversibly, whereas compound **30** affected the receptors only in a minor way. Scatchard plots of the saturation of [^{3}H]prazosin binding after preincubation with **31** indicated a decrease in the relative number of the sites (B_{max}) without a change in the affinity (K_D) of the remaining sites. Compound **31** retained the α_1 specificity of prazosin, since its affinity at [^{3}H]yohimbine binding sites on brain membranes was over 100 times lower than at [^{3}H]prazosin binding sites and **31** did not irreversibly block [^{3}H]yohimbine binding sites when preincubated with membranes.

Discussion

The covalent binding of reactive drugs to receptors may be expected to occur in two consecutive recognition steps, the first being reversible binding of the pharmacophore part of the drug to the binding site of the receptor followed by covalent bond formation between the reactive part of the drug with an adjacent site on the receptor, a process that leads to irreversible binding. These processes were clearly and thoroughly analyzed on opioid receptors.[22-24] The present results are in agreement with previously defined requirements: reactive drug must have high affinity for the receptor and the reactive residue of the drug must be able to reach a complementary group of the receptor. Nevertheless, the present series contains compounds that fulfill these requirements (e.g., **3** or **4**) but do not attach irreversibly to the receptor as compound **31** does. Perhaps the second recognition step also has an affinity component, and the chemoreactive group of the molecule must at first bond tightly and reversibly to a site close to the nucleophilic group of the receptor before the reaction may occur.

The irreversible activity of a reactive drug with receptor, which requires a second recognition step, may confer additional specificity to the process. This additional specificity was observed in an irreversible opioid antagonist,[22-24] but could not be found in alkylating β-blockers.[1-3] Careful investigations of compound **31** (Kusiak et al., manuscript submitted)[25] suggest that the use of compound **31** enables the delineation of a subtype of α_1-adrenoceptors. Compound **31**, under another name, SZL-49, was already partially evaluated on cells in vitro.[26]

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Experimental Section

Chemistry. Thin-layer chromatography was performed on precoated silica gel sheets (60F-254, 0.2 mm, EM Reagents). Silica gel grade H60, 230–400 mesh (Merck) was used for column chromatography. The structures of the compounds were verified in all cases by nuclear magnetic resonance spectra which were measured at 60 MHz on a JEOL PMX-60 spectrometer in deuteriochloroform or deuteriodimethyl sulfoxide with tetramethylsilane as the standard. Reliable empirical correlations of spectra with reaction products were possible since spectra of both starting components (for example, that of a starting acid and an amine for an amide) were available. Since no unusual features were observed, the spectra were not recorded here. Individuality (tested by thin-layer chromatography) and the correct ratio of starting components in the product (deduced from integrated nuclear magnetic resonance spectra) were the criteria used to confirm the proposed structures; elemental analyses were also obtained. Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected.

Preparation of Amides. A mixture of the respective carboxylic acid (0.25 mmol) and thionyl chloride (4 mL) was refluxed for 2 h and evaporated in vacuo and the residue twice coevaporated with toluene (4 mL). The acid chloride was then dissolved in dichloromethane (6 mL) and ethyl acetate (4 mL) and added to the stirred solution of 4-amino-6,7-dimethoxy-2-piperazin-1-yl)-quinazoline (in base form, 0.25 mmol) in dichloromethane (30 mL) and ethyl acetate (20 mL). The reaction mixture was stirred for 2 h and the reaction terminated by addition of saturated aqueous sodium bicarbonate solution. The product was extracted with dichloromethane, and the extracts were washed with water (3 × 10 mL), dried with magnesium sulfate, evaporated, and purified by column chromatography if required. Compound **13**, was not washed with sodium bicarbonate solution. Compound **27** was prepared by a direct reaction: the respective carboxylic acid (0.26 mmol) and 1,1'-carbonyldiimidazole (0.26 mmol) were dissolved in ethyl acetate (30 mL), and the solution was added dropwise with stirring to 4-amino-6,7-dimethoxy-2-(piperazin-1-yl)-quinazoline (0.25 mmol) in dichloromethane (30 mL) and ethyl acetate (20 mL). The mixture was stirred for 24 h and washed with saturated aqueous sodium bicarbonate solution and then with water, dried with magnesium sulfate, and evaporated.

Preparation of Thioureas or Ureas. The respective isothiocyanate (0.25 mmol) or isocyanate (0.25 mmol) was dissolved in dichloromethane (6 mL) and ethyl acetate (4 mL). This solution was added dropwise to the stirred mixture of 4-amino-6,7-dimethoxy-2-(piperazin-1-yl)quinazoline (0.25 mmol) in dichloromethane (30 mL) and ethyl acetate (20 mL).

Preparation of Isothiocyanates. A suspension of a salt of the respective amine (0.1 mmol) in ethyl acetate–dichloromethane (20 mL, 1:1) was stirred with an aqueous solution of sodium bicarbonate (1.0 mL, 1.0 mmol) for 1 h. Thiophosgene (1.2 mmol) was then added and the mixture stirred at room temperature for an additional 3 h. The organic layer was separated, washed with saturated aqueous sodium bicarbonate solution and water, dried with magnesium sulfate, and evaporated. The product was purified by crystallization or column chromatography.

4-Amino-2-[4-(bicyclo[2.2.2]octa-2,5-dien-2-ylcarbonyl)-piperazin-1-yl]-6,7-dimethoxyquinazoline (31). A mixture of 1,3-cyclohexadiene (3.9 g, 48.6 mmol) and propiolic acid (2.35 g, 33.5 mmol) was stirred at room temperature for 1 week. The mixture was then distilled and fraction 100–130 °C, 2.5 mmHg collected. Short column chromatography (160 g of silica gel) of this fraction using chloroform–methanol (98:2) as the eluent yielded bicyclo[2.2.2]octa-2,5-diene-2-carboxylic acid (3.8 g, 75%); mp 84–85 °C, R_f 0.29 in the same solvent (lit.[27] mp 78–80 °C).

The above acid (37.5 mg, 0.25 mmol) was refluxed with thionyl chloride (4 mL) for 2 h and evaporated in vacuo, and the residue was coevaporated two times with toluene (10 mL). The acid chloride thus obtained was then dissolved in dichloromethane (6 mL) and ethyl acetate (4 mL) and added to the stirred solution of 4-amino-6,7-dimethoxy-2-(piperazin-1-yl)quinazoline (base form, 73 mg, 0.25 mmol) which was dissolved in ethyl acetate (20 mL) and dichloromethane (30 mL). After 2 h the reaction mixture was washed with saturated aqueous sodium bicarbonate solution and then with water, dried with magnesium sulfate, and evaporated. The residue was recrystallized from ethyl acetate–methanol–cyclohexane, yielding compound **31** (91 mg, 85%); mp 265–267 °C; R_f 0.44 (dichloromethane–methanol, 9:1); ^{1}H NMR ($CDCl_3$) δ 6.85 and 6.82 (2 s, 2 H, aromatic protons), 6.43–6.22 (m, 3 H, vinyl protons), 5.41 (br s, 2 H, NH_2), 3.90–3.62 (m, 16 H, OCH_3 + piperidine protons + methine protons), 1.35 (s, 4 H, bridge protons). Anal. ($C_{23}H_{27}N_5O_3 \cdot 0.5CH_3OH$) C, H, N.

Pharmacology. Membrane Preparation. Rat brain cortices were homogenized in approximately 30 volumes/wet weight of 50 mM Tris buffer, pH 7.4, containing 10 mM $MgCl_2$ (this buffer was used throughout) by use of a Polytron (setting 6 for 10 s). The homogenate was centrifuged at 48000*g* for 10 min in a Sorvall RC-5B centrifuge. The resulting pellet was resuspended in buffer by use of a Polytron and recentrifuged. This process was repeated and the pellet was resuspended in approximately 200 volumes for affinity studies or 20 volumes for irreversibility experiments.

Irreversibility Experiments. Aliquots of a suspension of membranes (5 mL), prepared as described above, were incubated for 1 h at 30 °C with the addition of either only buffer (control) or a solution of the tested compound at 10–1000 nM concentration (experimental). The membranes were then diluted to approximately 40 mL with buffer and incubated a further 10 min on ice. The membranes were then washed by a series of four centrifugations and resuspensions before finally being resuspended in approximately 50 mL for assay of saturation of [^{3}H]prazosin binding. For isothiocyanates a buffer containing 50 mM phosphate and 1 mM ethylenediaminetetraacetate (pH 7) was used. Isothiocyanates were stable under conditions of the assay as follows from these findings: (a) thin-layer chromatography was used to establish that no decomposition occurred when compound **10** was incubated under conditions of assay (no membranes present); (b) hydrolysis of a model compound, phenyl isothiocyanate, was evaluated by spectrophotometry and utilizing buffer and temperature conditions of the assay. The expected linearity in graphs of the logarithm of percent phenyl isothiocyanate remaining versus time was confirmed, and from the graphs the half-lifes of phenyl isothiocyanate were calculated as 24 and 26.4 h when wavelengths of 272 and 266 nm were used, respectively; thus, only about 3% loss after 1 h, 30 °C assay may be expected.

Receptor Assay. [^{3}H]Prazosin binding sites were measured in an assay volume of 1 mL containing the following: [^{3}H]prazosin (~25 Ci/mmol, Amersham, 0.5 nM for affinity experiments, 0.05–1.0 nM, 12 different concentrations for saturation experiments); rat cortical membranes, 800 μL containing approximately 200–300 μg of membrane protein per assay; and buffer to make 1 mL. For affinity studies, tubes also contained prazosin derivatives (21 different concentrations between 1×10^{-12} and 1×10^{-5} M). Specific binding was defined as the difference between total binding (measured in the absence of any added ligand) and nonspecific binding (measured in the presence of 10 μM phentolamine). Duplicate tubes were used for each condition. Reaction tubes were incubated for 30 min at 30 °C, then diluted to 5 mL with ice-cold buffer, and filtered under reduced pressure through Whatman GF/C glass fiber filters. Tubes and filters were washed three additional times with 5 mL of buffer. Filters were placed in scintillation vials with 5 mL of Ready-Solv MP, and radioactivity was measured in a Beckman LS-5800 liquid scintillation system at ~45% efficiency.

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Registry No. 1, 19216-56-9; 2, 117040-02-5; 3, 117040-03-6; 4, 117040-04-7; 5, 117040-05-8; 6, 84050-50-0; 7, 70842-86-3; 8, 117067-19-3; 9, 117040-06-9; 10, 117040-07-0; 11, 117040-08-1; 12, 117040-09-2; 13, 117040-10-5; 13·HCl, 117040-11-6; 14, 80385-39-3; 15, 80385-35-9; 16, 117040-12-7; 17, 117040-13-8; 17·HCl, 117040-14-9; 18, 80385-57-5; 19, 117040-15-0; 20, 79221-43-5; 21, 79221-23-1; 22, 117040-16-1; 23, 117040-17-2; 24, 79221-26-4; 25, 117040-18-3; 26, 117040-19-4; 27, 117040-20-7; 28, 97131-41-4; 29, 117040-21-8; 30, 72898-23-8; 31, 107021-36-3; 4-amino-6,7-dimethoxy-2-(piperazin-1-yl)quinazoline, 60547-97-9; 1,3-cyclo-

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hexadiene, 592-57-4; propiolic acid, 471-25-0; bicyclo[2.2.2]octa-2,5-diene-2-carboxylic acid, 102589-30-0; 2-(chlorocarbonyl)bicyclo[2.2.2]octa-2,5-diene, 117040-22-9; (*E,E*)-ClC(O)CH=CHCH=CHC(O)OCH$_3$, 41967-17-3; (*E*)-ClC(O)CH=CH-*p*-C$_6$H$_4$F, 13565-08-7; ClC(O)CH=CH-*m*-C$_6$H$_4$F, 39098-87-8; ClC(O)CH=CH-*p*-C$_6$H$_4$NCS, 117201-50-0; (*E*)-ClC(O)CH=CH-*p*-C$_6$H$_4$NHC(O)CH$_2$Br, 117201-51-1; (*E,E*)-ClC(O)CH=CH-*p*-C$_6$H$_4$NHC(O)CH=CHC(O)OMe, 117226-09-2; ClC(O)CF=CHC$_6$H$_5$, 117201-52-2; (*E*)-ClC(O)CH=CHC(O)C$_6$H$_5$, 117201-53-3; *trans*-ClC(O)CHOCHC$_6$H$_5$, 76527-41-8; SCNC$_6$H$_{11}$, 1122-82-3; SCN-*p*-C$_6$H$_4$N$_3$, 74261-65-7; SCN-*p*-C$_6$H$_4$NHC(=S)NCH$_2$CH$_2$, 117201-54-4; ClC(O)-*m*-C$_6$H$_4$CH$_2$Cl, 63024-77-1; ClC(O)C$_6$H$_3$-*m*-I-*p*-NCS, 117201-55-5; (*E*)-ClC(O)(CH$_2$)$_4$-*p*-C$_6$H$_4$NHC(O)CH=CHC(O)OMe, 117201-56-6; 2-naphthalenecarbonyl chloride, 2243-83-6; 2-thiophenesulfonyl chloride, 16629-19-9.

Antiinflammatory 2,6-Di-*tert*-butyl-4-(2-arylethenyl)phenols

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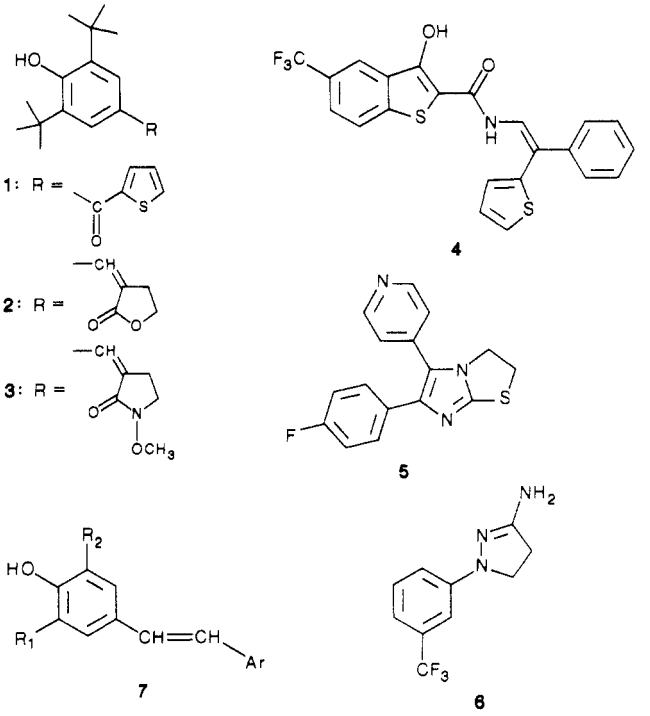
A series of 2,6-di-*tert*-butyl-4-(2-arylethenyl)phenols was prepared and examined for their ability to inhibit cyclooxygenase and 5-lipoxygenase in vitro and developing adjuvant arthritis in vivo in the rat. Structure–activity relationships are discussed. Among the best compounds is (*E*)-2,6-di-*tert*-butyl-4-[2-(3-pyridinyl)ethenyl]phenol (**7d**). It has an IC$_{50}$ of 0.67 μM for cyclooxygenase and 2.7 μM for 5-lipoxygenase and an ED$_{50}$ of 2.1 mg/kg in developing adjuvant arthritis. Additional in vivo data are reported for **7d**.

In an effort to obtain antiinflammatory drugs with a profile superior to cyclooxygenase-inhibiting nonsteroidal antiinflammatory drugs (NSAIDs), several groups have prepared dual cyclooxygenase/5-lipoxygenase (CO/LO) inhibitors. By blocking the production of prostaglandins from arachidonic acid, CO-inhibiting NSAIDs ameliorate the primary symptoms of arthritis via their analgesic, antipyretic, and antiinflammatory properties. However, they do not prevent tissue destruction or stop the disease process.[1]

Leukotrienes, the products of 5-LO metabolism of arachidonic acid, have been associated with immediate hypersensitivity reactions, anaphylaxis, and asthma.[2] 5-LO metabolites have also been linked to the inflammatory process. Leukotriene B$_4$ (LTB$_4$, 5,12-diHETE) has been shown to be a potent chemotactic substance for neutrophils.[3,4] In addition, products of 5-LO metabolism have been shown to stimulate neutrophil degranulation,[5] resulting in the release of lysosomal enzymes and reactive oxygen species, and to increase capillary permeability,[6] contributing to edema. These responses suggest a significant role in initiating and amplifying the inflammatory response. Therefore a dual CO/LO inhibitor would affect a wider range of proinflammatory mechanisms and presumably have an improved profile.

Among the dual CO/LO inhibitors reported are the three 2,6-di-*tert*-butylphenol derivatives R-830 (**1**),[7] KME-4 (**2**),[8] and E-5110 (**3**).[9] Other structurally unrelated dual CO/LO inhibitors include L-652,343 (**4**),[10] SK&F 86002 (**5**),[11] and one of the early compounds of this class, BW 755C (**6**).[12]

The design of the 2,6-di-*tert*-butylphenol derivatives was based on their potential antioxidant activity.[13] Stimulated polymorphonuclear leukocytes and macrophages release superoxide anion, which is metabolized to other reactive oxygen species including hydrogen peroxide, hydroxyl radical, and singlet oxygen. These reactive oxygen species are thought to contribute to the inflammatory process and tissue destruction.[14] While antioxidant activity has been demonstrated for **1** in autoxidation of peanut oil and FeCl$_2$-induced peroxidation of liposomes,[7] it is not established that any of the beneficial in vivo effects are brought about by scavenging reactive oxygen species released from stimulated cells.



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