Flavones. 2. Synthesis and Structure-Activity Relationship of Flavodilol and Its Analogues, a Novel Class of Antihypertensive Agents with Catecholamine Depleting Properties[1]

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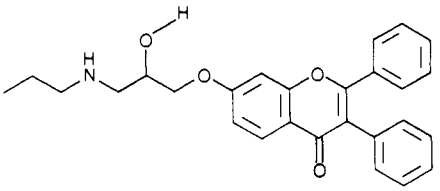
(3-Phenyl-7-flavonoxy)propanolamines have been shown to exhibit antihypertensive activity in spontaneously hypertensive rats. Although they are structurally similar to classical β-adrenergic blocking compounds, their activity is not due to inhibition of β-adrenoceptors. In the present study, a series of simple flavonoxypropanolamines was prepared to further explore the structural requirements for the antihypertensive effect of these compounds. A structure-activity relationship of these derivatives indicates that the position of the oxypropanolamine side chain, the hydroxy group of the side chain, steric bulkiness and length of N substituents, degree of the N-substitution, phenyl group at the 2-position of the chromone nucleus, and substituents of the phenyl group or B ring of the flavone play significant roles in imparting pharmacological effects. In addition, there is a good correlation between the antihypertensive activity and depletion of myocardial norepinephrine. Of these analogues tested, the most effective one was flavodilol (**3**). Only the 8-substituted analogue **6** was found to be a β-antagonist. Flavodilol was chosen for in-depth pharmacological, toxicological, and clinical evaluation.

New therapeutic approaches in the management of hypertension[2] have been directed toward modification of autonomic nervous activity by various agents such as dopamine receptor agonists,[3,4] inhibitors of dopamine β-hydroxylase,[5,6] and sympatholytics associated with catecholamine depletion.[7] Our recent research efforts in this regard have demonstrated that the (3-phenylflavonoxy)-propanolamine **1** lowers arterial blood pressure of spontaneously hypertensive rats (SHR) without antagonizing β-adrenergic receptors but with a substantial depletion of myocardial norepinephrine.[8] These results suggest that the antihypertensive activity of these 3-phenylflavones is related to modulation of sympathetic function by reducing noradrenergic neurotransmitter stores available for release during neuronal depolarization.[8]



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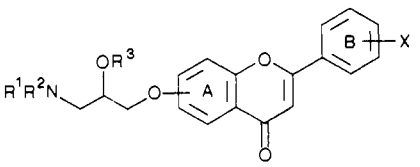

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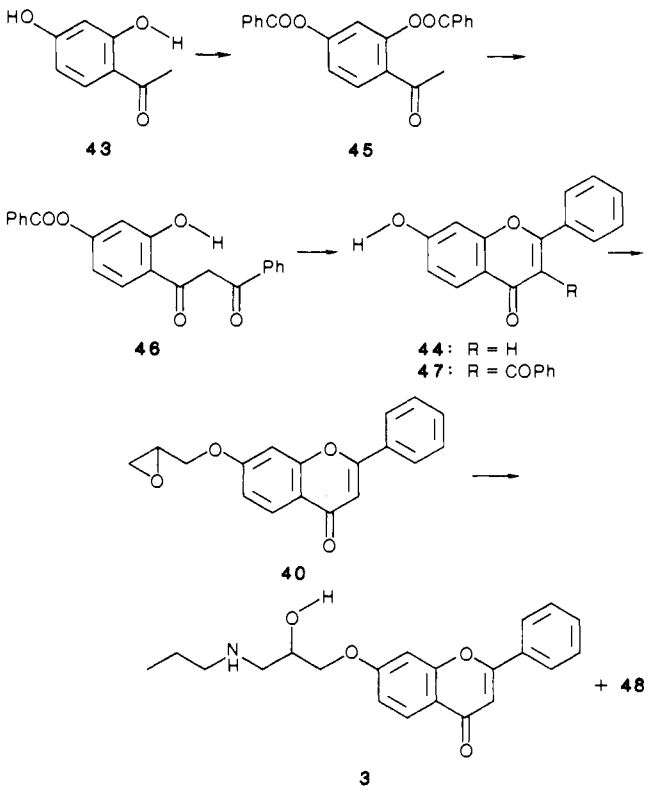
Compound **1** exhibited good activity but possessed several disadvantages including central nervous system (CNS) side effects. In the course of extending the work **2** was chosen to further explore and expand the structure-activity relationship. It was predicted that removal of the 3-phenyl group of **1** would result in a less lipophilic compound, **2**, than **1**.[9] As molecules with less lipophilicity would have less chance to cross the blood-brain barrier, **2** would produce less CNS side effects.

It is known that positional isomers often possess different pharmacological profiles. For example, propranolol, an α-isomer, and pronethalol, a β-isomer, are more potent than the corresponding β-isomer and α-isomer, respectively.[10] It was therefore anticipated that positional isomers of **2** would have different potencies and/or activities.

Scheme I



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(1) (a) Wu, E. S. C.; Cole, T. E.; Davidson, T. A.; Kinsolving, C. R.; Blosser, J. C.; Parker, R. B. *Pharmacologist* **1985**, *27*, 261. (b) Watkins, B. E.; Milgate, T. E.; Borrelli, A. R.; Kaiser, F. C.; Kinsolving, C. R.; Parker, R. B.; Wu, E. S. C. *Pharmacologist* **1985**, *27*, 261. (c) Wu, E. S. C.; Davidson, T. A.; Thomas, T. L.; Cole, T. E.; Loch, J. T.; Borrelli, A. R.; Kinsolving, C. R.; Parker, R. B. *Abstracts of Papers*, 190th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1985; MEDI 18. (d) Wu, E. S. C. U.S. Patent 4 495 198, 4 668 804.

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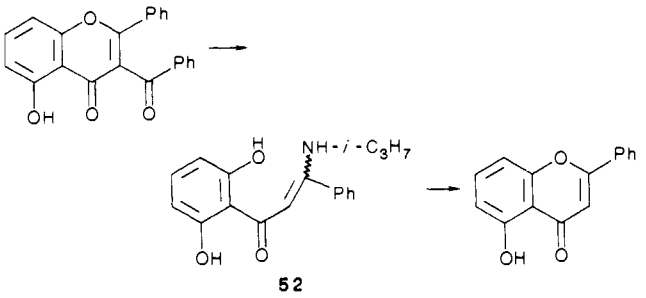
Scheme II



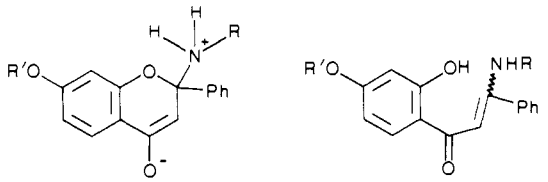
52

In this report we disclose the synthesis and antihypertensive activity of flavonoxypropanolamines **2** as part of our continuing research efforts to find a safe and effective antihypertensive drug.

Chemistry

Synthesis of flavodilol (**3**), as shown in Scheme I, represents a typical example of the general synthesis of flavonoxypropanolamines **2**. The Kostanecki reaction of resacetophenone (**43**), sodium benzoate, and benzoic anhydride failed to give 7-hydroxyflavone (**44**, R = H) in satisfactory yield as was claimed in the original work;[11] the major product in this reaction was 7-hydroxy-3-benzoylflavone (**17**). An alternative method via Baker–Venkataraman rearrangement of resacetophenone dibenzoate, which was prepared from resacetophenone and benzoyl chloride, to the diketone **46** followed by cyclization was employed to produce 7-hydroxyflavone[12] in good yield. Reaction of resacetophenone, benzoyl chloride, and potassium carbonate in refluxing acetone also led to the diketone **46** directly.[12b] The hydroxyflavone was allowed to react with 1 equiv of sodium hydroxide in aqueous ethanol and an excess of epichlorohydrin to yield the epoxide **40** as the major product.[13] Ring-opening of the epoxide **40** with *n*-propylamine in refluxing methanol afforded 7-[3-(*n*-propylamino)-2-hydroxypropoxy]flavone or flavodilol (**3**). As **3** reacted further with the epoxide **40** to give **48**, *N*,*N*-bis[3-(7-flavonoxy)-2-hydroxypropyl]-propylamine, it became necessary to use a large excess of amines to minimize this dialkylation reaction, which was also observed in the 3-phenylflavone series.[8]

In addition to the dialkylated side products, the β-aminochalcone **49** was also isolated.[15] The formation of **49** has been considered to proceed via Michael addition intermediate **50**. The yield of the chalcone depended on



50 **49**

R = $(CH_2)_2CH_3$; R′ = $CH_2CH(OH)NHR$

the quantity of the amine and the reaction time. However, the chalcone could be recyclized to flavodilol (**3**) in the presence of an acid.[16] The epoxide **40** was allowed to react with *N*-benzylpropylamine to give 7-[3-(*N*-benzyl-*N*-propylamino)-2-hydroxypropoxy]flavone (**56**), which was then reduced catalytically to flavodilol (**3**), to avoid using a large excess of primary amines and to minimize the Michael addition of the amine to the pyrone ring followed by pyrone ring-opening. Quaternization of **4** with iodomethane yielded **24**.

With the exception of 8-hydroxyflavone, most hydroxychromones reported in this paper were obtained by following literature procedures. Following the reported methods[17,18] for preparing 8-hydroxyflavone usually gave unidentifiable products. It was successfully synthesized via the Baker–Venkataraman rearrangement from 2,3-dihydroxyacetophenone in an overall yield of 39%. The required catechol was obtained in 89% yield from 2,3-dimethoxyacetophenone by demethylation with BBr_3.[19]

During the preparation of 5-hydroxyflavone from the corresponding diketone, 3-benzoyl-5-hydroxyflavone was formed as a major product.[20] Base-induced debenzoylation by sodium hydroxide, sodium carbonate, or potassium hydroxide failed. Upon being reacted with isopropylamine, 3-benzoyl-5-hydroxyflavone was converted into the chalcone **52**, a yellow solid, with concurrent loss of the benzoyl group[21] (Scheme II). The chalcone **52** was cyclized to 5-hydroxyflavone under acidic conditions.

The substituted phenyl analogues were obtained from resacetophenone and the appropriately substituted benzoic acid derivatives. Reduction of the 4′-nitro analogue **28** to **29** was effected by catalytic hydrogenation over 5% Pd/C.

As this work progressed, it became increasingly clear that the side chain, the 3-(alkylamino)-2-hydroxypropoxy moiety, played an important role in the pharmacological responses of these compounds. Two analogues, **36** and **37**, were synthesized to evaluate the significance of the hydroxy group of the side chain in imparting an antihypertensive effect. Alkylation of 7-hydroxyflavone with 3-

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(9) Using Chemlab-II Revision 8.0 from Molecular Design Ltd., partition coefficients (log P) of 1 and **3** (one of the positional isomers of **2**) in 1-octanol vs water were calculated to be 4.970 and 3.110, respectively.
(10) (a) Howe, R.; Crowther, A. F.; Stephenson, J. S.; Rao, B. S.; Smith, L. H. *J. Med. Chem.* **1968**, *11*, 1000. (b) Crowther, A. F.; Smith, L. H. *J. Med. Chem.* **1968**, *11*, 1009.
(11) (a) Robinson, R.; Venkataraman, K. *J. Chem. Soc.* **1926**, 2344; (b) *Org. React.* **1954**, 8, 128.
(12) (a) Baker, W. *J. Chem. Soc.* **1933**, 1381. (b) Jain, A. C.; Mathur, S. K., Seshadri, T. R. *J. Sci. Industr. Res.* **1962**, *21B*, 214.
(13) (a) In addition to the glycidyl ether,[14] this reaction also produced the chlorohydrin.[13b] (b) Wang, T.; Song, O. W.; Liu, H. Z. *Acta Pharm. Sin.* **1980**, *15*, 253.
(14) (a) Koda, A.; Sakamoto, K.; Nakamara, K.; Kano, T. *Jpn. J. Pharmacol.* **1980**, *30*, 559. (b) Koda, A.; Sakamoto, K.; Yanagihara, Y. *Jpn. J. Pharmacol.* **1980**, *30*, 437–447.
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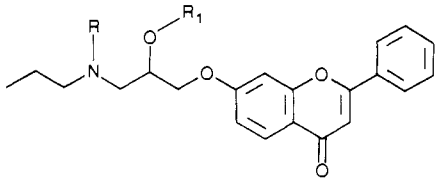
(20) Baker, W. *J. Chem. Soc.* **1934**, 1953.
(21) Similar observations were reported by Baker and Butt for the reaction of 3-acylchromones with benzylamine to give 2-[(β-benzylamino)crotonyl]phenol; Baker, W.; Butt, V. S. *J. Chem. Soc.* **1949**, 2142.

bromopropanol in K_2CO_3 and acetone gave 3-(7-flavonoxy)propanol (**53a**) (90% yield). Reacting with methanesulfonyl chloride and triethylamine in methylene chloride, the alcohol **53a** was transformed into the corresponding mesylate **53b**. The mesylate, without purification, was treated with isopropylamine in dimethyl sulfoxide at 80 °C for 20 h, or in hexamethylphosphoric triamide at 50 °C for 5 h, to yield the desoxy compound **36** (68% yield from **53a**).

The benzoate ester of flavodilol, **37**, was prepared by esterification of the carbamate analogue **51** followed by catalytic hydrogenation of the resulting ester **54** under acidic conditions.



	R	R^1
51	$PhCH_2OCO$	H
54	$PhCH_2OCO$	COPh

Synthesis of the flavanone derivative **41**, a reduced form of **4**, was initiated starting from 7-hydroxyflavanone to further study the importance of the pyrone ring. Reaction of 7-(2,3-epoxypropoxy)flavanone with isopropylamine produced **41** as well as a small amount of the corresponding chalcone **55**, a ring-opened form of **41**. Separation of these two isomers was not successful, as the former is base-labile whereas the latter is acid-labile.

Pharmacology

The antihypertensive activity of these compounds was evaluated in male spontaneously hypertensive rats (SHR) of the Wistar–Kyoto strain.[8] Arterial systolic blood pressure was measured by indirect tail cuff methods. Systolic blood pressure (SBP) was measured before and 7.5 h after each compound or its vehicle was administered orally to groups of SHR (N = 5 rats per treatment group). Changes in SBP by treatments were statistically evaluated by using a one-way analysis of variance and are expressed in Tables I–IV as a percentage decrease from pretreatment values.

Antagonism of β-adrenergic activity with these compounds was evaluated in anesthetized SHR instrumented with an arterial and venous catheter to measure blood pressure directly and to administer isoproterenol, respectively. The percentage inhibition of the depressor response of isoproterenol for the drug-treated group compared to vehicle-treated rats gauged the extent of in vivo β-adrenoceptor antagonism. In vitro assessments were also made of the affinity of test compounds for β-adrenergic receptors. Additional in vitro functional assay in guinea pig atria preparation was used to measure β-antagonist activity of test compounds by comparing the effects of different concentrations of the compounds on isoproterenol-induced contraction with those of propranolol.

Discussion

All of the compounds (Tables I–III) were assayed for their ability to lower blood pressure in SHR. In addition, most of them were examined for β-antagonist properties by measuring the in vivo β-adrenergic responses at a dose of 150 mg/kg, which was 2–6 times the minimally effective antihypertensive dose of these active compounds. Overall, the data from in vivo and in vitro assays suggest that these antihypertensive compounds have very weak or no β-antagonism,[22] despite the fact that they have the oxypropanolamine side chain, a side chain traditionally associated with β-antagonist activity. This correlates well with our previous findings in the (3-phenylflavonoxy)propanolamines.[8] Interestingly, a strikingly different profile was obtained for the 8-positional isomer (**6**). This compound showed unexpectedly good β-adrenoceptor inhibition while being devoid of antihypertensive activity in the SHR model employed.

A number of structure–activity relationships emerge from the data in Tables I–III. The greater activity of the straight N-alkyl analogues (**3** and **13**, Table I) and N-cycloalkyl analogues relative to their branched N-alkyl isomers (**4** and **14**, Table I) reveals the steric preference for a linear or cycloalkyl radical substituted on the nitrogen atom. There is an optimal chain length of three carbon atoms (**3** and **9**) in substituents of the secondary amine indicating the necessity of defined structural features in the drug–receptor interactions, as opposed to mere lipophilicity of N-substitution.[23] While secondary amines exhibit the optimal activity (**4** > **14**, **19**, **22**, **23**, and **24**), the primary amine (**12**) was inactive at 75 mg/kg (marginally active at 5 h after dosing). These results suggest that, in addition to hydrogen bonding with the NH group, optimal lipophilicity may be another important factor in the recognition site in eliciting antihypertensive activity. With regard to the nature of N substituents, there is a preference for alkyls over alkyls with heteroatoms (**17**, **20**, and **21**) and arylalkyls (**16**, **18**, and **20**).

The effect of the oxypropanolamine side chain at various positions of the A ring was also investigated. Compounds with the functionality at the 7-position (**3** and **4**) display better activity than the corresponding congeners at the 5-, 6-, and 8-position (**7**, **5**, and **6**, respectively). As mentioned earlier the 8-positonal isomer **6** is devoid of antihypertensive activity but is very potent in inhibiting β-receptors both in in vitro and in vivo tests. These facts may indicate that the oxypropanolamine side chain of **3** adapts an extended conformation such as **56**[37] and fits into the active sites of the receptors, thus triggering the biological responses. On the other hand the side chain of **6** may stretch out from the 8-position and lean toward the γ-pyrone as shown in **57**, a conformation similar to the X-ray structures of β-blockers such as butocrolol (**58**)[24] and propranolol.[25] This conformation may well explain the difference of biological profiles of **3** and **4** from those of **6** and propranolol. While the side-chain conformation **59** of **5** can overlap somewhat with **56**, the side chain of **7** cannot attain a conformation superimposable with that of **56**. Introduction of a polar group such as a hydroxyl into the A ring produces a compound (**8**) with weak activity.

In general, modification of the 2-hydroxyl group of the oxypropanolamine side chain results in significant loss of the antihypertensive activity. The ester analogue **37** is substantially less active than the parent compound **13**. Replacement of the hydroxyl with a hydrogen atom (**36**) produces a loss of activity. These results clearly indicate

(22) Using a published procedure,[8] affinity for β-adrenergic receptors of **3**, **4**, **5**, **6**, **9**, **10**, **14**, **15**, and *l*-propranolol were measured and their inhibition constants are 4.0, 12, 2.2, 7.5×10^{-3}, 1.5, 7.5, 18, 5.5, and 1.6×10^{-3} μM, respectively.

(23) This view is further supported in the 3-phenylflavones series where N-*n*-pentyl and N-*n*-hexyl analogues are inactive at 75 mg/kg.[8] In the same series, the N-cyclooctyl analogue is also inactive at 75 mg/kg (unpublished result).

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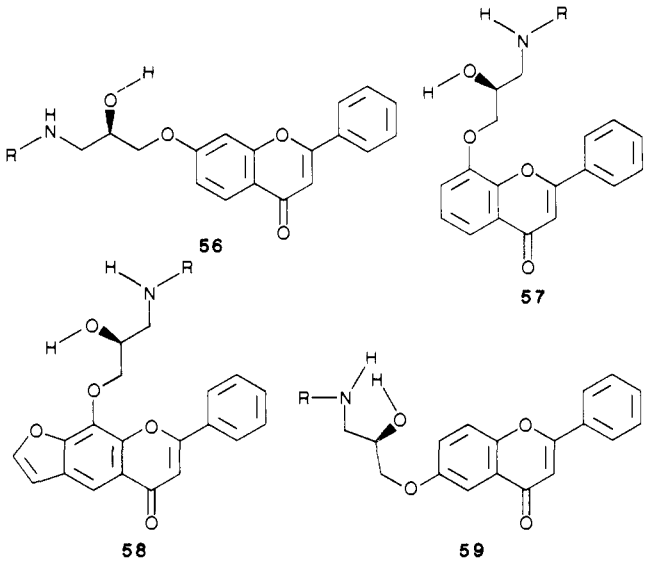


the critical role played by the hydroxyl function, presumably serving as a hydrogen bonding donor or acceptor.

The fourth region of modification was the B ring (Table II). Substitution of the para position of the B ring with a variety of substituents, i.e., NO_2 (**28**), NH_2 (**29**), OCH_3 (**30**), and CH_3 (**31**),[35] causes a loss of activity. However, placement of a chlorine atom at the para or ortho position (**25**, **26**) produces compounds whose activity does not vary appreciably from that of the corresponding parent **4**. The inactivity of the *m*-chloro derivative **27** and compounds **28**, **29**, **30**, and **31** reflects the preference for a unperturbed π-electron system, as further supported by λ_{max} of their UV spectra (λ_{max} in MeOH for **4**, **25**, **26**, **27**, **28**, **29**, **30**, and **31** are 304, 305, 296, 307, 314, 307 and 365, 307 and 322, 308, respectively). Replacement of the para hydrogen with a methyl group (**31**) results in a compound devoid of activity, suggesting bulk intolerance in this region.

Certain structural modifications were also made on the γ-pyrone ring (Tables II and III). Compared to parent compounds **3** and **4**, the 3-methyl[26] (**32**) and 3-benzyl (**33** and **34**) analogues are relatively weak in activity. Insertion of an oxygen atom between C-3 and the methyl group, **35**, of **32** results in a loss of activity. These results demonstrate the importance of the steric bulkiness at this position as well as a preference for carbon over oxygen. The inactivity of the 2-methyl (**38**, Table III) derivative clearly points out that the 2-phenyl group is essential for activity. The flavanone analogue **41** was prepared to test if the planar structure of the chromone ring or the α,β-unsaturation is needed for activity. Compound **41** was found to be inactive, thus suggesting that a planar structure might be required for activity. For further SAR information, intermediates **39** and **40** were evaluated and found to be inactive.

Most of the compounds synthesized in this series are structurally related to β-blockers. The compounds were subjected to a functional in vivo β-receptor antagonist assay to exclude the possibility of β-antagonism being part of the mechanisms responsible for their antihypertensive action. At a dose of 150 mg/kg, all the compounds except compound **6** and propranolol did not block isoproterenol responses in rats. Compound **6** inhibited the depressor response of isoproterenol at 20 mg/kg, po, which is comparable to the blockade by propranolol (**42**) at 10 mg/kg, po.[27] Some cursory results from a functional in vitro β-receptor antagonist assay in guinea pig atria also indicated that **3**, **4**, **5**, and **32** exhibited only a slight inhibition of the isoproterenol response at a dose of 1×10^{-4} M, which is approximately 10^{-3} times that of propranolol. The inhibition constants of **3**, **4**, **5**, **6**, **9**, **10**, **14**, **15**, and **38** were measured in a β-receptor radioligand binding assay to further characterize the lack of β-adrenoceptor antagonism. Inhibition constants except that of **6** were all 3 to 4 orders of magnitude greater than that of propranolol, suggesting that these compounds have very weak antagonist properties. Similar results were observed by Wang and his co-workers[13b,28] and Da Re.[26] They reported that **4**,[28] **5**,[28] **14**,[28] the N-isopropyl analogue[28] of **7** and **32**[26] did not exhibit β-blocking activities.

Catecholamine Depletion and Antihypertensive Effect. In search of the mechanism by which these compounds reduce blood pressure, the effects of some representative compounds from this series and related compounds on biogenic amine levels in peripheral tissues and brain were studied. Table IV summarizes some important changes of certain biogenic amines in tissues that may play an important role in regulating blood pressure. Depletion of biogenic amines varied considerably among tissues and specific amines were subject to differential depletion. There were marked effects on heart norepinephrine (NE) levels in SHR upon oral administration of these flavone analogues. Moderate change was seen in heart serotonin (5-HT) levels. Catecholamines, NE and dopamine (DA), in brain were marginally decreased. Slight reduction of epinephrine (EPI) in adrenal stores occurred. On chronic treatment for 4 days, **60** completely depleted adrenal stores of EPI. In the acute treatment norepinephrine levels in heart were much more sensitive than those in brain or hypothalamus. This selectivity may reflect pharmacokinetic data showing a very limited ability of these compounds to penetrate the blood–brain barrier.[30]

At the effective antihypertensive doses (17.5, 35, and 75 mg/kg) of **3** there were depletions of 79–94%, 49–63%, 6–19%, and 31–38% in heart NE, heart 5-HT, and hypothalamus NE and DA, respectively, 5 h after dosing. A substantial decrease in biogenic amine levels occurred in these tissues between the inactive antihypertensive dose (8 mg/kg) and the marginally active one (17.5 mg/kg). As the dose increased to 35 and 75 mg/kg, the increment in antihypertensive activity was associated with further small incremental decreases of NE in heart but not in brain. With a 61% reduction of heart NE and 29% in 5-HT at a dose of 75 mg, **35** did not decrease blood pressure. However, at 150 mg/kg **35** demonstrated a marginally antihypertensive effect (16% reduction) as well as a 70% depletion of heart NE and a 37% depletion of heart 5-HT. These data plus those of **3** suggest that decreases in blood pressure are accompanied by only extensive depletion of peripheral NE levels. Although there were 37% and 47% reductions of NE levels and DA levels, respectively, in hypothalamus, which were higher than those of **3** and **35**

(26) Da Re, P.; Primofiore, G. P.; Bertelli, A. *J. Med. Chem.* **1972**, *15*, 868.

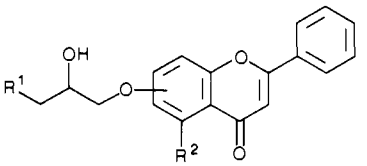
(27) Propranolol, at the dose of 1 mg/kg, iv, produced essentially the same antagonistic effects as those observed with compound **6** at 1 mg/kg, iv, in the anesthetized dog.

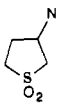
(28) After this work was completed, scientists at Tianjin, China, reported that several flavone analogues including **4**, **5**, the isopropyl derivative of **7**, and **14** exhibited no significant β-antagonism.[13b]

(29) *Clin. Pharm. Therap.* **1983**, *33*, 191.

(30) (a) Strand, J. C.; Watkins, B. E.; Parker, R. B.; Wu, E. S. C.; Milgate, T. E.; Borrelli, A. R.; Kaiser, F. C., submitted to *J. Cardiovasc. Pharmacol.* (b) Blosser, J. C.; McCreedy, S.; Parker, R. B.; Kinsolving, C. R.; Watkins, B. E.; Wu, E. S. C., submitted to *J. Cardiovasc. Pharmacol.*

Table I. Flavonoxypropanolamines[a]

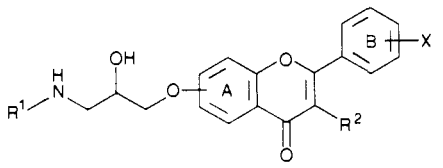


compd	position	R^1	R^2	mp,[b] °C	recrystllzn solvent	formula	anal.[c]	SBP[d] pretreatment value ± SE	SBP[d] dose, mg/kg, po	SBP[d] % decrease at 7.5 h
3[e,f]	7	n-C_3H_7HN	H	171–172	MeOH	$C_{21}H_{23}NO_4 \cdot C_4H_4O_4$	C, H, N	199 ± 5	17	15
								196 ± 4.7	35	27
								203 ± 2.7	75	32
4[e,f]	7	i-C_3H_7NH	H	230–323	MeOH–ether	$C_{21}H_{23}NO_4 \cdot HCl$	C, H, N, Cl	203 ± 2.3	17	NS
								204 ± 8.9	35	21
								208 ± 11.4	75	27
5[e,f]	6	i-C_3H_7NH	H	209–211	i-PrOH–BuOH	$C_{21}H_{23}NO_4 \cdot HCl$	C, H, N, Cl	199 ± 3.1	75	12
6[g]	8	i-C_3H_7NH	H	225–227	MeOH	$C_{21}H_{23}NO_4 \cdot HCl$	C, H, N, Cl	209 ± 2.02	75	NS
7[i]	5	n-C_3H_7NH	H	194–195	CH_2Cl_2–i-PrOH	$C_{21}H_{23}NO_4 \cdot HCl$	C, H, N, Cl	157 ± 4	30 (iv)	NS
8	7	i-C_3H_7NH	OH	236–237	i-PrOH	$C_{21}H_{23}NO_5 \cdot HCl$	C, H, N, Cl	195 ± 2.6	75	15
9[e,f]	7	c-C_3H_5NH	H	214–215	MeOH–ether	$C_{21}H_{21}NO_4 \cdot HCl$	C, H, N, Cl	197 ± 2.9	17	21
								198 ± 3.9	35	31
								196 ± 5.2	75	32
10[f]	7	C_2H_5NH	H	244–246	MeOH	$C_{20}H_{21}NO_4 \cdot HCl$	C, H, N, Cl	186 ± 3.0	35	16
11	7	CH_3NH	H	251–252	MeOH	$C_{19}H_{19}NO_4 \cdot HCl \cdot {}^1/_2H_2O$	C, H, N, Cl	190 ± 2.8	35	NS
12	7	H_2N	H	191 ± 192	MeOH	$C_{18}H_{17}NO_4 \cdot HCl$	C, H, N, Cl	187 ± 2.3	75	NS
13	7	n-C_4H_9NH	H	224–225	MeOH	$C_{22}H_{25}NO_4 \cdot HCl$	C, H, N, Cl	202 ± 4.5	35	26
14[e,f]	7	t-C_4H_9NH	H	202–204	MeOH–ether	$C_{22}H_{25}NO_4 \cdot HCl$	C, H, N, Cl	201 ± 7.4	150	NS
15[f]	7	c-$C_6H_{11}NH$	H	242–243	MeOH	$C_{24}H_{27}NO_4 \cdot HCl \cdot {}^1/_2H_2O$	C, H, N, Cl	201 ± 6.0	35	20
16	7	$C_6H_5CH_2NH$	H	233–234	MeOH–ether	$C_{25}H_{23}NO_4 \cdot HCl$	C, H, N, Cl	223 ± 2.2	75	NS
17	7	HOC_2H_4NH	H	205–207	MeOH	$C_{20}H_{21}NO_5 \cdot HCl$	C, H, N, Cl	189 ± 2.2	75	NS
18	7	3,4-$(MeO)_2$Ph-C_2H_4NH	H	231–233	MeOH	$C_{28}H_{20}NO_6 \cdot HCl$	C, H, N, Cl	204 ± 5.1	75	NS
19	7	$(CH_3)_2N$	H	116–118	EtOH	$C_{20}H_{22}NO_4 \cdot HCl$	C, H, N, Cl	207 ± 5.6	75	−11 (NS)
20	7	$PhOCH_2CH(OH)CH_2NH$	H	209–211	MeOH	$C_{27}H_{27}NO_6 \cdot HCl$	C, H, N, Cl	198 ± 8.7	75	NS
21	7		H	232–234	DMF	$C_{22}H_{23}NO_6S \cdot HCl$	C, H, N, Cl, S	196 ± 3.8	75	NS
22	7	$PhN(CH_2CH_2)_2N$	H	175–177	$CHCl_3$–MeOH	$C_{28}H_{28}N_2O_4$	C, H, N	217 ± 2.4	75	NS
23	7	$PhCH_2(i\text{-}C_3H_7)N$	H	190–191	MeOH–ether	$C_{28}H_{29}NO_4 \cdot HCl$	C, H, N, Cl	200 ± 6.3	75	NS
24	7	i-$C_3H_7(CH_3)_2NI$	H	133–135	EtOH	$C_{23}H_{28}NO_4I$	H, N, I; C[h]	200 ± 4.8	75	NS

[a] All the compounds in this table were tested in isoproterenol-response inhibition. Inhibition of the depressor response of isoproterenol (0.25 μg/kg, iv) was determined in SHR. Four male SHR per group were used. Dose administered (with free base factor corrections) was 150 mg/kg po. Most of the compounds tested did not show inhibition except 6 and propranolol which exhibited 100% inhibition. [b] Melting point of free bases, HCl salts, or maleate salt as indicated in the formulas. [c] Elemental analyses were within 0.4% of theory unless otherwise noted. [d] Five male rats per dosage group. Percentage falls in systolic blood pressure were recorded at the indicated hour after dosing. Values in the table are statistically significant ($p > 0.05$) relative to control values; NS indicates that percentage falls in systolic blood pressure were not statistically significant. Reduction of systolic blood pressure by this series of compounds usually reaches maximum at 5–7 h after dosing. [e] In guinea pig atria assay of six compounds tested, 3, 4, 5, 9, 14, and 32, only 3, 4, 5, and 12 exhibited a slight inhibition of the isoproterenol response at a dose of 1×10^{-4} M, which is approximately 1000 times that of propranolol (1×10^{-7}). [f] The inhibition constants, (K_i), obtained from β-adrenergic receptor binding assay are 4.0, 12, 2.2, 7.5×10^{-3}, 1.5, 7.5, 18, 5.5, 23, and 1.6×10^{-3} μM for 3, 4, 5, 6, 9, 10, 14, 15, 38, and l-propranolol, respectively. [g] The lowest dose at which 6 blocks the response of isoproterenol in rats was 15 mg/kg, compared to 10 mg/kg for propranolol hydrochloride. In anesthetized dogs, 6 and propranolol hydrochloride were equally effective at 1 mg/kg, iv. [h] C: calcd, 53.58; found, 53.12; the sample had 5.97% of ethanol and 0.97% of water. [i] This compound was not tested in isoproterenol-response inhibition.

at antihypertensive doses, 19 at 150 mg/kg did not produce any significant decrease in blood pressure. Results from 19 together with those from 4 and 1 indicate that changes in blood pressure were not dependent on any meaningful changes in catecholamine contents in brain or adrenal tissues, despite the fact that measurements of blood

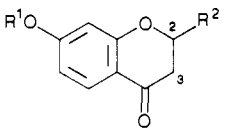
Table II. 7-Flavonoxypropanolamines with Substituents on the B Ring[a-e]



compd	R^1	R^2	X	mp,[b] °C	recrystllzn solvent	formula	anal.[c]	SBP[d] pretreatment value ± SE	SBP[d] dose, mg/kg, po	SBP[d] % decrease at 7.5 h
25	i-C_3H_7	H	p-Cl	258–259	DMF	$C_{21}H_{22}ClNO_4$·HCl	C, H, N, Cl	192 ± 4.4	35	19
26	i-C_3H_7	H	o-Cl	114–117	DMF	$C_{21}H_{22}ClNO_4$·HCl	C, H, N, Cl	192 ± 4.3	35	18
27	i-C_3H_7	H	m-Cl	277–279	DMF	$C_{21}H_{22}ClNO_4$·HCl	C, H, N, Cl	200 ± 4.2	75	NS
28	i-C_3H_7	H	p-NO_2	213–215	EtOH	$C_{21}H_{22}N_2O_6$	C, H, N	202 ± 1.0	75	NS
29	i-C_3H_7	H	p-NH_2	264–266	i-PrOH	$C_{21}H_{24}N_2NO_4$·2HCl·$^1/_2H_2O$	H, N, Cl; C[f]	206 ± 1.0	65	NS
30	i-C_3H_7	H	p-OCH_3	234–235	n-BuOH	$C_{22}H_{25}NO_5$·HCl	C, H, N, Cl	198 ± 3.8	75	10 (NS)
31	i-C_3H_7	H	p-CH_3	243–244	n-BuOH	$C_{22}H_{25}NO_4$·HCl	C, H, N, Cl	196 ± 4.5	75	NS
32[e]	i-C_3H_7	CH_3	H	120–122	EtOH	$C_{22}H_{25}NO_4$·HCl	C, H, N, Cl	206 ± 3.6	35	10
								208 ± 3.8	75	27
33	i-C_3H_7	$PhCH_2$	H	174–176	i-PrOH	$C_{28}H_{29}NO_4$·$C_4H_4O_4$	C, H, N	200 ± 3.9	75	NS
34	n-C_3H_7	$PhCH_2$	H	196–198	i-PrOH	$C_{28}H_{29}NO_4$·HCl	C, H, N, Cl	198 ± 4.3	75	14
35	i-C_3H_3	CH_3O	H	172–174	MeOH–ether	$C_{22}H_{25}NO_5$·HCl	C, H, N, Cl	194 ± 5.1	75	NS

[a-e] Table I. [f] C = calcd, 55.81; found, 56.39. This sample contained 1.71% of water.

Table III. Miscellaneous 7-Substituted Chromones[a-f]



compd	R^1	R^2	C_2–C_3	mp,[b] °C	recrystllzn solvent	formula	anal.[c]	SBP[d] pretreatment value ± SE	SBP[d] dose, mg/kg, po	SBP[d] % decrease at 7.5 h
36	i-$C_3H_7NH(CH_2)_3$	Ph	C=CH	248–249	EtOH	$C_{21}H_{23}NO_3$·HCl	C, H, N, Cl	204 ± 4.4	75	NS
37[g]	n-$C_3H_7NHCH_2CH(O_2CPh)CH_2$	Ph	C=CH	158–160	i-PrOH	$C_{28}H_{27}NO_5$·HCl	H, N, Cl; C[h]	187 ± 1.3	75	22.5
38	i-$C_3H_7NHCH_2CH(OH)CH_2$	CH_3	C=CH	174–176	MeOH–ether	$C_{16}H_{21}NO_4$·HCl	C, H, N, Cl	196 ± 6.3	75	NS
39	H	Ph	C=CH					217 ± 2.8	75	NS
40	OCH_2CHCH_2	Ph	C=CH	134–135	CH_2Cl_2	$C_{18}H_{14}O_4$	C, H, O	120 ± 2.3	75	NS
41	i-$C_3H_7NHCH_2CH(OH)CH_2$	Ph	$CHCH_2$	117–119	MeOH–ether	$C_{21}H_{25}NO_4$[i]	C, N; N[j]	198 ± 2.6	75	NS
42[e,f]	propranolol							212 ± 3.0	75	NS

[a-f] Table I. [g] This compound was not tested in isoproterenol-response inhibition. [h] C: calcd, 68.08; found, 67.62. [i] A small amount of the corresponding chalcone was present in this compound. [j] H: calcd, 7.08; found, 6.44.

pressure in the time-dependent study were not done at the same time as those of biogenic amine contents (see footnote *f* of Table IV).

Thus, reductions of heart 5-HT, brain or hypothalamus NE and DA, and adrenal EPI did not appear to be associated with blood pressure lowering effects of **1**, **4**, **19**, **35**, and **60**. A common denominator for exhibiting antihypertensive activity seems to reside in extensive depletion (i.e., greater than 70% depletion) of heart NE.[30b] A time-dependent study of **4** at a dose of 150 mg/kg also lends further support to the concept that a threshold of 70% depletion of peripheral NE levels is needed for reducing blood pressure by these compounds.

Flavodilol

Results from these studies led to the selection of flavodilol[29] (**3**) for in depth pharmacological, toxicological, and clinical evaluation. In conscious SHR, flavodilol produced a dose-related blood pressure reduction of up to 30% and decreased heart rate up to 10–15%. Optimal blood pressure lowering effects were reached 5–8 h after dosing. A transient vasodilatory effect was also observed. The mechanism for the antihypertensive activity of flavodilol can be attributed, at least in part, to depletion of catecholamines in heart and vascular tissues. Details of these experiments will be published elsewhere.[30]

Experimental Section

The melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were determined in the indicated solvent on a Varian EM360A NMR spectrometer at the ambient operating temperature with tetramethylsilane as internal standard for proton spectra unless otherwise stated. Chemical shifts are given in ppm units, and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m multiplet. Infrared spectra were recorded on a Nicolet MX-1 Fourier transform infrared spectrophotometer. Elemental analyses were performed by Galbraith laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical value when indicated by symbols of the element unless otherwise noted.

Table IV. Effects of Oral Administration with Flavone Derivatives on Systolic Blood Pressure and Biogenic Amines Levels in Heart, Hypothalamus or Brain, and Adrenal of SHR[j]

			% decrease					
				heart		hypothalamus (H) or brain (B)[c]		adrenal
compd	mg/kg, po	h[a]	SBP	NE	5-HT	NE	DA	EPI
3[b]	4	5	7*	21*	−10	2(H)	4	NA
	8	5	3	38*	11	0(H)	−6	NA
	17.5	5	6[d]	79*	49*	6(H)	31*	NA
	35	5	22*	89*	61*	19*(H)	37*	NA
	75	5	31*	94*	63*	15*(H)	38*	NA
19[b]	75	5	0	18	0	20*(H)	29*	NA
	150	5	5	7	0	37*(H)	47*	NA
35[b]	35	5	0	55*	7	12(H)	29*	NA
	75	5	4	61*	29*	16*(H)	26*	NA
	150	5	16*	70*	37*	28*(H)	37*	NA
4[j]	35	24	−4	57*	NA	−4(B)	Na	17*
	75	24	16*	100[e]	NA	2(B)	NA	23*
	150	1	NA[f]	40*	NA	0(B)	NA	10
	150	3	NA[f]	71*	NA	2(B)	NA	4
	150	7	NA[f]	100[e]	NA	10*(B)	NA	16
	150	24	33*	100[e]	NA	14*(B)	NA	39*
1[j]	35	24	26*	100[e]	NA	12*(B)	NA	10
60[g]	150[h]	24[h]	34*	100[e]	NA	37*(B)	NA	100[i]

[a] Hours post dose when animals were sacrificed and the systolic blood pressure was measured. [b] Control values of SBP (pretreatment values), NE and 5-HT in heart, and NE and DA in hypothalamus of **3**, **19**, and **35** are 192 ± 4 (mmHg), 4.2 ± 0.5 (ng/mg protein), 0.59 ± 0.05 (ng/mg protein), 14.5 ± 1.3 (ng/mg protein), and 3.8 ± 0.4 (ng/mg protein), respectively. Biogenic amine contents were determined by reverse phase liquid chromatography. [c] Brain refers to whole brain minus cerebellum. [d] Although the decrease in SBP in this particular experiment was relatively low (6% only), the data was proven to be statistically significant. The average decrease (5 h post dose) of SBP by **3** at the dose of 17.5 mg/kg was 17% from control. Since 17.5 mg/kg is a threshold dose to effect the antihypertensive activity, variable results did occur at this dose. [e] NE was not detectable; the detectable limit for heart is 0.15 μg/g tissue. [f] The change in blood pressure of SHR was not measured in this particular study. However, in this series of compounds the onset of activity generally started at 2 h after dosing and reached peak effects at 5–7 h post dose. In a separate study a 20% decrease of SBP at 2 h, 22% at 5 h, 26% at 7.5 h, and 24% at 24 h after dosing occurred when 150 mg/kg of **4** was administered. [g] 7-[2-Hydroxy-3-(isopropylamino)propoxy]isoflavone. [h] Animals were dosed for 4 consecutive days, when SBP was measured and animals were sacrificed 24 h after the last dosing. Control values for SBP, NE in heart and in brain, and EPI were 208 ± 3.8 (mmHg), 0.380 ± 0.038 (μg/g tissue), 0.221 ± 0.005 (μg/g tissue), and 0.139 ± 0.009 (μg/g tissue), respectively. Biogenic amine contents were determined by fluorometric assay. [i] EPI was not detectable. [j] Control values of SBP (pretreatment values), NE in heart, NE in brain, and EPI in adrenal of **4** and **1** are 201 ± 3.8 (mmHg), 0.357 ± 0.15 (μg/g tissue), 0.190 ± 0.004 (μg/g tissue), and 397 ± 28 (μg/g tissue), respectively. Biogenic amine contents were determined by fluorometric assay. [k] NA = not available. Asterisk indicates significantly different from control, $p < 0.05$, Anova and Newman Kuel's t-test.

Resacetophenone Dibenzoate (45). To a solution of resacetophenone (499.3 g, 3.29 mol), pyridine (1579 mL, 19.70 mol) and anhydrous ether (1642 mL) at 0 °C (ice bath) was added dropwise benzoyl chloride (953 mL, 1154 g, 8.21 mol) over 1 h. A precipitate was immediately formed when benzoyl chloride was added and then it became a very thick suspension. After the addition, the mixture was stirred at 0 °C for 1 h and then room temperature for 1 h. The solid was collected by filtration and washed with 3300 mL of ether (anhydrous). The filtrate was washed with water, 10% aqueous HCl, 10% aqueous $NaCHO_3$, and saturated brine solution and dried ($MgSO_4$). Crystals were formed while drying. The filtered solution was evaporated, leaving an orange-brown semisolid, which crystallized on standing, 778.2 g, mp 80–81 °C. Solid (crystals and $MgSO_4$) from the filtration was suspended in 1.5 L of $CHCl_3$, stirred for about 30 min, and filtered. The filtrate, upon evaporation, afforded white crystals, 389.5 g, mp 80–81 °C (lit.[12] mp 80–81 °C). The two crops were combined: 1167.7 g, yield 98.5%.

[4-(Benzoyloxy)-2-hydroxybenzoyl]benzoylmethane (46). (a) A suspension of resacetophenone dibenzoate (1021 g, 2.83 mol) and powdered anhydrous potassium carbonate (587 g, 4.25 mol) in acetone (5660 mL) was mechanically stirred and heated under reflux in the presence of nitrogen. As the reaction proceeded, the mixture turned into a fluffy, yellow suspension. It was monitored by TLC (70% ether in hexane) and usually required 2 days before the starting dibenzoate disappeared. After cooling, the yellow solid was collected by filtration and washed with 5 L of toluene and then with 4 L of water. The solid (sodium salt of the dibenzoylmethane) was stirred with 10% aqueous HCl (2 L) for about 30 min, collected by filtration, and rinsed with water. The desired product, 764.8 g, (75% yield), mp 167–69 °C (lit.[12] mp 167 °C), was obtained. This process usually afforded the dibenzoylmethane in 70–79% yield.

The organic layers (acetone solution and toluene washing) were combined and evaporated to yield a brown gum, which crystallized overnight. The brown crystals were washed with toluene (ca. 100 mL), 10% HCl (ca. 100 mL), water (ca. 200 mL), and anhydrous ether to give nice crystals, 191.7 g, mp 179 °C dec. When subjected to cyclization–debenzoylation conditions (cf. experiment for synthesis of 7-hydroxyflavone), the brown crystals produced dark brown prisms, 74.1 g, mp 266–269 °C (MeOH), mmp with 3-benzoyl-7-hydroxyflavone 265 °C; its NMR and R_f value (in 90% ether/hexane) were the same as those of 3-benzoyl-7-hydroxyflavone. A mixture of 7-hydroxyflavone and 3-benzoyl-7-hydroxyflavone (14.5 g) was obtained from neutralizing the aqueous washing of the yellow solid of the diketone salt.

(b) A mixture of benzoyl chloride (53 mL, 0.456 mol), resacetophenone (32 g, 0.21 mL), powdered anhydrous potassium carbonate (131 g), and acetone (600 mL) was stirred and heated to reflux. The reaction was monitored by TLC for completion and then cooled to room temperature. The solid was collected and washed with acetone (150 mL). The yellow solid was washed with water until the filtrate was neutral, 47.7 g (63%), mp 162–167 °C.

7-Hydroxyflavone. (a) A yellow suspension of [4-(benzoyloxy)-2-hydroxybenzoyl]benzoylmethane (764.8 g, 2.12 mol), glacial acetic acid (7571 mL), and anhydrous sodium acetate (1590 g, 19.4 mol) was gently refluxed under nitrogen. As soon as the reflux started, the suspension turned into a brown solution, which was refluxed overnight (16 h). TLC (90% ether in hexane) showed the complete cyclization and hydrolysis. The resulting mixture was cooled and water added until no further white precipitate was formed. The white light needles were obtained by filtration; 419.4 g, mp 242–243 °C (lit.[11] mp 240 °C), 85% yield.

(b) A mixture of resacetophenone (5 g, 33 mmol), benzoic anhydride (60 g, 133 mmol), and sodium benzoate (65 g, 225 mmol) was heated at 180–190 °C for 6 h. After cooling, the pale brown mass was broken up and alcohol (95%, 160 mL) was added to the reaction mixture. The mixture was boiled during the gradual addition of potassium hydroxide (18 g), dissolved in water (20

mL), and then heated for 30 min at reflux. After removal of the greater part of the alcohol by distillation, the residue was dissolved in water and filtered off. The filtered solution was saturated with carbon dioxide and the precipitated light brown, crude product was isolated (7.5 g, mp 198–210 °C). Recrystallization from acetic acid (charcoal) and water afforded 1.20 g (6%) of 3-benzoyl-7-hydroxyflavone, mp 268 °C (lit.[23] mp 265 °C). Anal. ($C_{22}H_{14}O_4$) C, H, N. The mother liquor was evaporated and the residue was recrystallized from acetic acid and water to give 0.61 g of yellow crystals, mp 210–215 °C; thin layer chromatography (silica gel, in 80% ether in hexane) indicated that the solid was a mixture of 7-hydroxyflavone and 3-benzoyl-7-hydroxyflavone.

7-(2,3-Epoxypropoxy)flavone (40). To a solution of 82.2 g (2.06 mol) of sodium hydroxide in 585 mL of water were added 3.7 L of 2-propanol and then 490 g (2.06 mol) of 7-hydroxyflavone. To the above mixture was then added 1645 mL (20.5 mol) of epichlorohydrin, and the mixture was heated at 70 °C for 2 h with stirring. The hot reaction mixture was filtered to remove a dimeric byproduct (a glycidyl ether[13a]). The filtrate was concentrated under reduced pressure (water aspirator) at 50 to 60 °C. The semisolid residue was treated with 4.4 L of refluxing 2-propanol and more of the dimer was filtered off from the hot mixture. The clear filtrate, on cooling, yielded a solid. This was collected, washed with 600 mL of 2-propanol, and air-dried; yield 434.3 g (72%) of a tan-colored product, mp 123–130 °C. It was purified by high pressure liquid chromatography to give white needles, mp 134–135 °C: IR (KBr) 1630, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) 2.66–3.10 (m, CH_2 in the epoxide ring), 3.20–3.50 (m, 1 H, CH in the epoxide ring), 4.05 (dd, J = 11, 6 Hz, 1 H, OC*H*H), 4.41 (dd, J = 11, 3 Hz, 1 H, OCH*H*) 6.70 (s, 1 H, C_3H), 6.80–8.20 (m, 8 H, Ar H). Anal. ($C_{18}H_{14}O_4$) C, H. Small amounts of 7-(3-chloro-2-hydroxypropoxy)flavone[13b] and 1,3-bis(7-flavonyl)glycidyl ether[14] were also isolated with a yield of 2–5% for each product.

7-[3-(Propylamino)-2-hydroxypropoxy]flavone (3). (a) To 1.03 L (12.6 mol) of *n*-propylamine were added 53 g (0.18 mol) of 7-(2,3-epoxypropoxy)flavone and 540 mL of absolute ethanol. The mixture was heated at 50 to 55 °C for 1.5 h, with stirring. The reaction mixture was clarified by filtration and the filtrate concentrated on a Rotovap, under reduced pressure, to a volume of 400 mL. The product was filtered and washed with 100 mL of absolute ethanol, yielding 38.3 g (60%) of a bright yellow solid, mp 144–146 °C.

The crude base was suspended in a mixture of 341 mL of water and 114 mL of 95% ethanol and then treated with 54 mL of 2.5 N hydrochloric acid to obtain a solution of pH 1 to 2. The aqueous solution was then clarified by filtration. Water (114 mL) was added to the filtrate, followed by the slow addition of approximately 54 mL of 0.5 N NaOH until the mixture was tested for a pH of 10 to 11. The mixture was stirred for $^1/_2$ h. The product was filtered, washed with 268 mL water, and air-dried; yield 36.2 g (57%) of a pale yellow powder, mp 145–147 °C: IR (KBr) 3300, 3120, 1690, 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) 0.96 (t, J = 7.3 Hz, 3 H, CH_3), 1.56 (qt, J = 7.3, 7.3 Hz, 2 H, CH_2CH_3), 2.64 (dt, J = 12.2, 7.3 Hz, 1 H, CH_2CHHN), 2.68 (dt, J = 12.2, 7.3 Hz, 1 H, CH_2CHHN), 2.81 [dd, J = 12.2, 7.3 Hz, 1 H, CH_2CH CH(OH)], 2.93 [dd, J = 12.2, 3.5 Hz, 1 H, $HCHCH(OH)$], 4.12 [dd, obscured by CH_2O, 1 H, $CH(OH)$], 4.12 (s, 2 H, CH_2O), 6.78 (s, 1 H, C_3H), 7.01–7.03 (m, 2 H, Ar protons), 7.52–7.54 (m, 3 H, Ar protons), 8.15 (d, J = 9.5 Hz, 1 H, C_5H); MS, m/e 354.1705 (M + 1), $C_{21}H_{24}NO_4$ required 354.1704. Anal. ($C_{21}H_{23}NO_4$) C, H, N.

To 51.0 g (0.144 mol) of 7-[3-(propylamino)-2-hydroxypropoxy]flavone in 1.2 L of absolute ethanol was added 20.7 g (0.178 mol) of maleic acid. The mixture was heated to reflux to obtain a solution, and then 10.5 g of Nuchar C-190-N was added. After a few minutes, the hot mixture was filtered and the filtrate was allowed to cool with stirring. The product was collected and washed with 400 mL of absolute ethanol. The damp product was dissolved in 1.5 L of refluxing absolute ethanol, the hot solution treated with 10.5 g of Nuchar C-190-N, and the mixture filtered. The filtrate was allowed to cool to 23 °C with stirring. The product was collected, washed with 250 mL of absolute ethanol, air-dried overnight, and then dried under vacuum at room temperature for 48 h; yield, 47.2 g (70%) of a colorless maleate salt, mp 171–172 °C. Anal. ($C_{25}H_{27}NO_8$) C, H, N.

(b) 7-[2-Hydroxy-3-(*N*-benzyl-*N*-propylamino)propoxy]flavone (0.88 g, 2 mmol), which was similarly prepared as shown in a, was dissolved in 5 mL of acetic acid with heat. Methanol (10 mL) and 10% Pd/C (0.20 g) were added under nitrogen to the acetic acid solution, and the mixture was hydrogenated in a Parr hydrogenator until 1 equiv of hydrogen had been absorbed. The reaction mixture was filtered, and the filtrate was evaporated to give 0.61 g (86%) of white crystals, mp 140–142 °C.

2′,4′-Dihydroxy-β-(isopropylamino)chalcone. A solution of 3-benzoyl-7-hydroxyflavone (1.37 g, 4 mmol), isopropylamine (11.8 g, 200 mmol), and 2-propanol (8 mL) was refluxed overnight. The low boiling liquids were taken off and the residual viscous liquid slowly crystallized as yellow prisms. Recrystallization from methanol gave yellow prisms, 0.71 g (60%); mp 136–138 °C: ^1H NMR ($CDCl_3$) 1.25 (d, J = 6 Hz, 6 H, two CH_3), 3.50 (br s, 4 H, NHCH, two OH), 5.55 (s, 1 H, HCCPh), 6.45 (m, 2 H, H ortho to OH), 7.50 (br s, 6 H, Ar protons). Anal. ($C_{18}H_{19}NO_3$) C, H, N.

7-[3-(*N*-Isopropyl-*N*-methylamino)-2-hydroxypropoxy]flavone Methiodide (24). A mixture of the free base 4 (2.0 g, 5.7 mmol), anhydrous potassium carbonate (2.0 g, 14.5 mmol), methyl iodide (4.56 g, 32 mmol), and acetone (20 mL) was refluxed under nitrogen for 19 h. The white solid was collected and washed with a small amount of absolute ethanol; 4.87 g of the solid was recrystallized from isopropyl alcohol (70 mL) to give 2.70 g of the crude methiodide, mp 143 °C dec. Further recrystallization from absolute EtOH gave 2.50 g of pale yellow prisms, mp 133–135 °C dec. Anal. ($C_{23}H_{28}NO_4I$) C, H, N, I (adjusted for 0.12% 2-propanol and 5.9% ethanol).

2,3-Dihydroxyacetophenone. A solution of boron tribromide (108.87 g, 435 mmol) in 145 mL of methylene chloride was slowly added to a cooled (−78 °C) solution of 2,3-dimethoxyacetophenone[17] in methylene chloride (290 mL) under nitrogen. The cooling bath was removed and the resulting dark orange solution was slowly warmed up to room temperature and stirred for 1.5 h. The dark orange suspension was then poured into ice–water (1 L) and filtered to remove the dark red solid. The aqueous layer was separated out and extracted with chloroform (800 mL) twice. The combined organic extracts were washed with water and then saturated brine solution and dried (Na_2SO_4), thus giving 24.12 g (89%) of brown crystals, mp 96–97 °C (lit.[31] mp 97–98 °C).

7-[2-Hydroxy-3-[*N*-propyl-*N*-[(benzyloxy)carbonyl]amino]propoxy]flavone (51). Triethylamine (6.07 g, 60 mmol) was added slowly to a white suspension of 5.08 g (15 mmol) of flavodilol (3), 7-[2-hydroxy-3-(propylamino)propoxy]flavone, and benzyl chloroformate (2.69 g, 15 mmol) in 100 mL of methylene chloride. The resulting solution was refluxed for 4 h. A white solid was formed and removed by filtration; the filtrate was diluted with CH_2Cl_2 (150 mL), washed with 5% aqueous HCl several times to remove a trace amount of 3 and water and dried (Na_2SO_4). Evaporation of the solvent gave a tan viscous liquid, which solidified upon addition of ether. Recrystallization of the solid (6.68 g), mp 90–92 °C, from EtAc–hexane afforded 3.70 g (52% yield) of white crystals, mp 92–94 °C. Anal. ($C_{29}H_{29}NO_6$) C, H, N.

7-[2-(Benzoyloxy)-3-[*N*-propyl-*N*-[(benzyloxy)carbonyl]amino]propoxy]flavone (54). A mixture of the crude flavodilol carbamate (14.2 g, 30 mmol), benzoyl chloride (6.33 g, 45 mmol), and triethylamine (12.14 g, 80 mmol) in CH_2Cl_2 (200 mL) was stirred at room temperature under nitrogen overnight. TLC indicated that the reaction was complete. The reaction mixture was diluted with methylene chloride (~300 mL) and washed twice with sodium bicarbonate and once with water. The dried (K_2CO_3) solution, upon evaporation, gave 15.52 g of brown crystals, mp 122–124 °C. Recrystallization from isopropyl alcohol gave light yellow prisms (10.7 g, 56% yield), mp 126–127 °C. Anal. ($C_{36}H_{33}N_7$) C, H, N.

7-[2-(Benzoyloxy)-3-(propylamino)propoxy]flavone (37). A solution of flavodilol carbamate benzoate (8.0 g, 13.5 mol) in 60 mL of acetic acid and 60 mL of absolute ethanol was Parr hydrogenated in the presence of 1.0 g of 10% Pd/C until the theoretical amount of hydrogen was taken up. The reaction mixture was filtered through a glass-wool filter paper and the

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filtrate was evaporated. Toluene was added to remove a small amount of acetic acid. The residue was dissolved in ~40 mL of *i*-PrOH and acidified with HCl/EtOH. Anhydrous ether was added to afford 6.04 g (91% yield) of pale yellow crystals, mp 158–160 °C (*i*-PrOH–Ether). Anal. ($C_{28}H_{27}NO_5$·HCl) C, H, N, Cl.

7-[3-(Isopropylamino)propoxy]flavone Hydrochloride (36). (a) 7-[3-[(Methylsulfonyl)oxy]propoxy]flavone (53b). To a yellow suspension of 7-(3-hydroxypropoxy)flavone[34] (81.49 g, 275 mmol) and triethylamine (83.48 g, 825 mmol) in methylene chloride (550 mL) at 0 °C was added dropwise methanesulfonyl chloride (63.0 g, 550 mmol). The resulting mixture was stirred at 0 °C for 1 h and then warmed up to room temperature for 1 h. The reaction mixture was poured into 500 g of ice water to decompose the excess of methanesulfonyl chloride. The aqueous layer was separated from the organic layer and extracted with methylene chloride (2 × 100 mL). The organic layers were combined, washed with water and saturated brine solution, and dried ($MgSO_4$). The dried solution, upon evaporation, gave 76.54 g (76% yield) of a beige solid, mp 125–128 °C.

(b) 7-[3-(Isopropylamino)propoxy]flavone Hydrochloride (36). A mixture of the mesylate **53b** (14 g, 37.4 mmol), isopropylamine (11.1 g, 187 mmol), and DMSO (75 mL) was heated at 80 °C for 19 h and cooled to room temperature. Isopropylamine was evaporate and water was added to precipitate out the product as yellow crystals; 11.56 g, mp 89–91 °C. The free base was dissolved in 300 mL of isopropyl alcohol with heat and filtered. The filtrate was acidified with saturated HCl/EtOH. The yellow crystals formed, 9.16 g, mp 226–228 °C, were collected. The crystals were recrystallized from ethanol, mp 248–249 °C. Anal. ($C_{21}H_{23}NO_3$·HCl) C, H, N, Cl.

Indirect Blood Pressure Measurement in Unanesthetized Rats. Male spontaneously hypertensive rats (SHR) of the Wistar–Kyoto strain were used. Arterial systolic blood pressure was measured with an indirect tail cuff method. Prior to each set of measurements, the rats, 5 per treatment group, were heated in an environmental chamber at 35–37 °C for 15–20 min to raise body temperature sufficiently to allow the pulse in the dilated caudal artery to be easily detected. In all cases, at least three consecutive measurements were obtained, and the average was reported as the systolic blood pressure for a particular rat at a specific time. Pretreatment measurements were made in the afternoon before an experiment that began on the following morning. Only rats with a systolic pressure of 180 mmHg or higher were used. All doses of test compounds administered were corrected to deliver 100% of the free base. Test compounds were suspended in a 4% aqueous Clearjel solution, adjusted to a volume of 1% body weight. Statistical analysis of the results was performed by using analysis of variance.

Isoproterenol-Response Inhibition. SHR, four animals in each treatment group, were dosed orally[38] with 150 mg/kg of the various test compounds, as a suspension in 4% Clearjel. Four hours after dosing, generally the time of near-optimal antihypertensive response of active compounds, the rats were anesthetized with sodium pentobarbital (45 mg/kg, ip), and the left femoral artery and vein were cannulated. Arterial pressure was then continually monitored from the arterial cannula. The acute vasodepressor response, and occasionally tachycardiac response, was assessed following intravenous administration of 0.25 μg/kg of isoproterenol in 0.1 mL of saline. Potential ability of a test compound to inhibit isoproterenol responses was evaluated by comparison to responses of rats that had been treated orally with the Clearjel vehicle.

In vivo assessment of possible antihypertensive mechanisms of active compounds must be made at the time of appreciable blood pressure reduction, which in this instance was 4–5 h after oral dosing. This consideration is essential to allow sufficient time for adequate oral absorption of compounds and consequent onset of pharmacological effects.[38]

Guinea Pig Atria Preparation. Guinea pig atria were excised and hung in a 50-mL tissue bath containing Krebs' physiological solution, which consists of 113 mM NaCl, 4.7 mM KCl, 2.5 mM calcium lactate, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11.6 mM glucose. The solution was constantly bubbled with a gas mixture of 95% O_2 and 5% CO_2 at 37 °C. Following a 30-min equilibration period, the tissue was challenged with 2×10^{-8} M isoproterenol and the increase in heart rate and force of contraction was recorded with a Grass FT.03 transducer attached to a Beckman polygraph. The effects of different concentrations of the compounds on heart rate and force of contraction were compared with those of isoproterenol to determine possible β-adrenergic agonist properties. Antagonist activity was measured by comparing the effects of different concentrations of the compounds on isoproterenol-induced contraction with those of propranolol.

Biogenic Amine Content. Biogenic amine levels were quantified by using two procedures, namely, (a) reverse phase liquid chromatography with electrochemical detection (HPLC) and (b) fluorospectrometry.

(a) HPLC. Rats were decapitated and tissues quickly removed, weighed, and frozen in a dry ice/acetone bath. Heart, adrenal, and spleen tissue can be stored at −20 °C for 2 weeks and brain tissue for 1 month without significant degradation of amines. All analyses were performed within this time frame. Aorta tissue was assayed within 2 days after freezing. Biogenic amines were extracted from tissues that were thawed in 0.1 M perchloric acid containing 2 mM sodium heptane sulfonate and 0.1 mM Na_2EDTA (10 volumes/g wet weight of heart, spleen or adrenal, 5 volumes/g wet weight of brain tissue, 2 volumes/g wet weight of aorta) and homogenized for 30 s at 4 °C by using a Brinkman Instruments Polytron at medium speed. Homogenates were centrifuged at 25000 × *g* for 15 min and the pellet was discarded. The supernate was filtered through Amicon CF50 membrane cones by centrifugation at 2000 × *g* for 20 min at 4 °C and filtrates were either analyzed immediately or frozen overnight at −20 °C.

Biogenic amines were chromatographed on a DuPont C18 column (25 cm × 4.1 mm id) using a mobile phase consisting of 90% phosphate buffer (0.07 M KH_2PO_4, 0.1 mM Na_2EDTA, and 2 mM sodium heptane sulfonate), 6% methanol, and 4% acetonitrile at a flow rate of 1 mL/min at ambient temperature. *N*-Methyldopamine, an internal standard for HPLC quantification, was routinely added (10 μL of a 0.004% solution per 2 mL of homogenization buffer) at the time of tissue homogenation. *N*-Methyldopamine was also added to known amounts of biogenic amine standards to identify retention times and to construct standard curves for each biogenic amine. Amines were detected on a Bioanalytical System LC-4 amperometric detector and quantified with the aid of a 3392 Hewlett-Packard integrator. Protein concentrations were determined by the method developed by Lowry et al.[32]

(b) Fluorospectrometry. For fluorometric analysis, tissues were extracted according to the procedures described by Welch and Welch.[33] Tissues were homogenized in 10 volumes of 0.01 N HCl at 4 °C. Three-milliliter aliquots were mixed with 10 mL of butanol, 0.1 mL of 10% EDTA, and 2 g of NaCl by vigorous shaking for 10 min. Following centrifugation at 1000 × *g* for 10 min, the butanol phase was transferred to 50-mL conical centrifuge tubes containing 20 mL of washed heptane (i.e., pre-extracted with one-fifth volume of 1 N NaOH and 1 N HCl) and 1.5 mL of 0.1 M $NaKHPO_4$ buffer, pH 6.5. Tubes were shaken and centrifuged as described above and 0.5-mL aliquots of the buffer phase were taken for biogenic amine analysis. Derivatization and fluorometric analysis for NE and EPI were performed according to the methods described by Laverty and Taylor.[34] NE and EPI were measured with an Aminco SPF500 fluorospectrometer (excitation/emission wavelengths: 400 nm/480 nm for NE and

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(38) The reason that test compounds were orally administered instead of iv was to simulate the conditions used in the indirect blood pressure measurement in unanesthetized SHR.

410 nm/500 nm for DA) and compared with standard curves prepared daily for each catecholamine. Sensitivity was 0.5 ng for NE and 4 ng for EPI, values that were approximately twice that of the blank.

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Registry No. **3**, 79619-31-1; **3**·$C_4H_4O_4$, 79619-32-2; **4**, 84858-20-8; **4**·HCl, 76323-05-2; **5**, 117096-72-7; **5**·HCl, 76323-03-0; **6**, 117096-73-8; **6**·HCl, 117096-95-4; **7**, 117096-74-9; **7**·HCl, 117096-96-5; **8**, 117096-75-0; **8**·HCl, 84858-43-5; **9**, 87272-34-2; **9**·HCl, 84858-30-0; **10**, 84858-33-3; **10**·HCl, 84858-32-2; **11**, 84858-58-2; **11**·HCl, 84858-31-1; **12**, 84858-59-3; **12**·HCl, 84858-39-9; **13**, 84858-35-5; **13**·HCl, 84858-34-4; **14**, 117096-76-1; **14**·HCl, 76323-06-3; **15**, 84858-37-7; **15**·HCl, 84858-36-6; **16**, 117096-77-2; **16**·HCl, 117096-97-6; **17**, 117096-78-3; **17**·HCl, 117096-98-7; **18**, 117096-79-4; **18**·HCl, 117096-99-8; **19**, 117096-80-7; **19**·HCl, 117097-00-4; **20**, 117096-81-8; **20**·HCl, 117097-01-5; **21**, 117096-82-9; **21**·HCl, 117097-02-6; **22**, 117096-83-0; **23**, 117096-65-8; **23**·HCl, 117097-03-7; **24**, 117096-64-7; **25**, 117096-84-1; **25**·HCl, 84858-27-5; **26**, 117096-85-2; **26**·HCl, 84858-25-3; **27**, 117096-86-3; **27**·HCl, 117097-04-8; **28**, 117096-87-4; **29**, 117096-88-5; **29**·2HCl, 117097-05-9; **30**, 117096-89-6; **30**·HCl, 117097-06-0; **31**, 117096-90-9; **31**·HCl, 106287-81-4; **32**, 37933-65-6; **32**·HCl, 38186-01-5; **33**, 117096-91-0; **33**·$C_4H_4O_4$, 117097-07-1; **34**, 117096-92-1; **34**·HCl, 84858-41-3; **35**, 117096-93-2; **35**·HCl, 117097-08-2; **36**, 117096-70-5; **36**·HCl, 117096-71-6; **37**, 117096-68-1; **37**·HCl, 117097-09-3; **38**, 81716-13-4; **38**·HCl, 117097-10-6; **39**, 6665-86-7; **40**, 84858-19-5; **41**, 117096-94-3; **42**, 525-66-6; **43**, 89-84-9; **45**, 66832-97-1; **46**, 5465-06-5; **47**, 117096-61-4; **49** (R′ = H), 117096-63-6; **51**, 117096-66-9; **53a**, 93876-03-0; **53b**, 117096-69-2; **54**, 117096-67-0; **56**, 117096-62-5; 2,3-$(CH_3O)_2C_6H_3COCH_3$, 38480-94-3; 2,3-$(OH)_2C_6H_3COCH_3$, 13494-10-5; epichlorohydrin, 106-89-8; norepinephrine, 51-41-2.

Phenolic Metabolites of Clomiphene: [(*E*,*Z*)-2-[4-(1,2-Diphenyl-2-chlorovinyl)phenoxy]ethyl]diethylamine. Preparation, Electrophilicity, and Effects in MCF 7 Breast Cancer Cells

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The triarylethylene antiestrogen clomiphene (**1**) was previously shown to undergo biotransformation to an active metabolite, 4-hydroxyclomiphene (**2**), and to 3-methoxy-4-hydroxyclomiphene (**3**) plus the respective regioisomers of these, **4** and **5**. We now report the synthesis and further chemical and biochemical studies on **3**–**5**. Coupling of 4-[2-(diethylamino)ethoxy]benzophenone (**10**) with either 4-(benzyloxy)benzaldehyde (**11a**) or its 3-methoxy analogue **11b** in the presence of titanium, followed by chlorination and deprotection of the intermediate triarylethylenes, gave **4** and **5**, respectively. Condensation of benzylmagnesium chloride with the (2-methoxyethoxy)methyl (MEM) ether of 4-[2-(diethylamino)ethoxy]-3′-methoxy-4′-hydroxybenzophenone (**8**), followed by mild acid treatment, afforded deschloro **3** due to facile MEM ether hydrolysis. Acetylation of this, followed by chlorination and deacetylation, gave **3**. Compounds **4** and **5** reacted readily with nucleophiles. In particular, 2-mercaptoethanol reacted with **4** to afford deschloro vinyl thioether **13** as suggested by NMR spectral studies, a result that implicated allene–quinone **14** as the electrophilic species produced in solution from **4**. Antiestrogen binding sites and estrogen receptors from MCF 7 human breast cancer cells interacted with **3** and **5** with affinities comparable to those of tamoxifen and **1**, respectively; **5** was shown not to bind irreversibly with these sites. Inhibition of MCF 7 cell proliferation by **3**–**5** at 5 μM concentrations (76%, 57%, and 49%, respectively, relative to drug-free controls) compared favorably to that observed with 5 μM **1** (80%). These results suggest that **3**–**5** as well as **2** may contribute to the antiestrogenic effects of **1**.

Triarylethylene antiestrogens have been used successfully in breast cancer treatment.[1] In experimental studies the antiestrogen clomiphene (**1**)[2] has been shown to suppress the proliferation of cultured human breast cancer cells and to inhibit the growth of chemically induced breast cancer in the rat.[3] Also, clomiphene has been suggested to antagonize estrogen-mediated feedback inhibition of gonadotropin-releasing hormone secretion in the hypothalamus, a mechanism that may account for its ability to induce ovulation.[4]

Interaction with drug-metabolizing enzymes may be a complicating factor in elucidating the molecular mechanism(s) of action of **1**. In the presence of rat liver microsomes **1** underwent N-oxidation, N-deethylation, and conversion to **2**.[5a] The first two metabolites had estrogen receptor (ER) affinities similar to that of **1**, and **2** had much higher ER affinity and greater antiestrogenic and antiproliferative potencies than did **1**.[3,5] Further studies of the metabolism of **1** in the immature female rat have

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