increasing concentrations of the test compounds (10^{-4}–10^{-9} M), in triplicate, and fixed concentration of ^{3}H-E_2 (5×10^{-9} M). Each incubate (70 μL) in TEA buffer (Tris-HCl, 10 mmol; ethylenediaminetetraacetic acid (EDTA), 165 mmol; NaN_3, 0.02%, pH 7.4) was 7% in dimethylformamide (DMF). Free and bound ^{3}H-E_2 were seaprated by treating each incubate with 10 μL of charcoal–dextran slurry (2.5 and 0.25% v/v, respectively) in TEA buffer for 20 min. Radioactivity of 50-μL aliquot of each incubate was measured in minivials containing 5 mL of scintillation fluid (1.5:2.5:2.5, v/v mixture of methanol–dioxane–toluene containing 0.5% PPO (2,5-diphenyloxazole), 0.01% POPOP [1,4-bis(5-phenyloxazol-2-yl)benzene], and 9% naphthalane).

The competition experiments with liver microsomal fraction, using ^{3}H-Tam as the reference ligand, were performed according to previously reported procedure.[25] The liver microsomal fractions were first incubated at 4 °C for 2 h, with 2 μM diethylstibestrol added in a small volume of DMF to saturate ER sites. Aliquots (200 μL) of the fraction were then mixed in a Pyrex glass tube with 20 μL of competitor (1×10^{-9} M to 3×10^{-6} M) and 20 μL of ^{3}H-Tam (1×10^{-9} M) dissolved in 35% DMF–TEA buffer. The tubes were incubated for 18 h at 4 °C and then treated with charcoal–dextran slurry (100 μL) for 15 min at 4 °C to separate bound and free ^{3}H-Tam. The tubes were centrifuged at 1000*g* for 15 min, and the supernatants were counted for radio activity.

Uterotrophic and Antiuterophic Activity. For uterotrophic activity various doses of the test compounds, suspended in 0.1 mL of propylene glycol–0.9% saline (1:1, v/v), were injected subcutaneously to the test animals on three consecutive days, while the control group of animals received the vehicle alone. Antiuterotrophic assay was similarly performed by administering various doses of test compounds and 0.3 μg of E_2 in the case of rats and 0.1 μg of E_2 in the case of mice, each suspended in 0.1 mL of propylene glycol–0.9% saline (1:1, v/v), to the test animals a two different sites, while the control group of animals received the injection of E_2 and the vehicle alone. Animals were autopsied 24 h after last injection and their uterine weights were recorded in the usual manner.

Acknowledgment. We acknowledge Dr. Nitya Anand for his keen interest in this study. We are grateful to Dr. A. H. Todd of ICI Ltd., U.K. for providing a gift sample of tamoxifen and to Dr. William R. Fields of Eli Lilly, for providing a gift sample of LY-117018. We would also like to acknowledge the Department of Science and Technology, Government of India, for financial support in carrying out the AEBS affinity studies, which were undertaken at IIT, Bombay.

Structure–Activity Relationship of Antiestrogens. Phenolic Analogues of 2,3-Diaryl-2*H*-1-benzopyrans†

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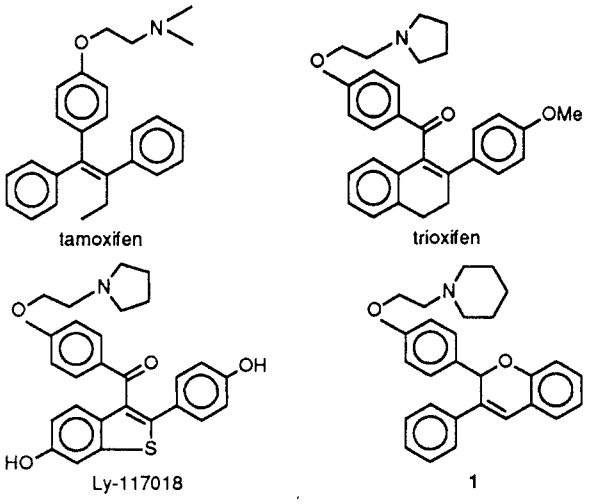
Phenolic analogues of 2-[4-(2-piperidinoethoxy)phenyl]-3-phenyl-2*H*-1-benzopyran (1), a novel antiestrogen, were synthesized and evaluated for their structure–activity relationship. Incorporation of OH at position 7 was found to improve receptor affinity of the benzopyran while having no effect on its action as an antagonist. Similar substitution of 2-phenyl as well potentiated receptor affinity as well as antagonist activity of the prototype. The monophenol **19** and the diphenol **25** were thus found to be good receptor ligands, devoid of estrogen agonist activity and associated with marked antiestrogenic activity of comparable order. Both caused nearly complete inhibition of the estradiol stimulated uterine growth in rats as well as mice and were thus found to be better antiestrogens than tamoxifen, trioxifen, and LY-117018. A binding-site model for estrogen receptor rationalizing the structure–activity relationship of benzopyrans in relation to that of the triarylethylene and the triarylpropenone antiestrogens has been discussed.

Introduction

The triarylethylene (TAE) and (*Z*)-1,2,3-triarylpropen-1-one (TAP) antiestrogens, represented by tamoxifen, trioxifen, and LY-117018 (Chart I), are well recognized as being partial agonists–antagonists.[1-6] Our continuing efforts to study structure–activity relationship (SAR) of antiestrogens,[7-13] so as to unravel the molecular origins of their partial agonist character, resulted recently in the development of 2,3-diaryl-2*H*-1-benzopyran (DABP) as yet another group of potent antiestrogens.[12,13] The ensuing studies on SAR resulted in 2-[4-(2-piperidinoethoxy)-phenyl]-3-phenyl-2*H*-1-benzopyran (1) (Chart I) as a potent antiestrogen possessing weaker agonist character than tamoxifen as well as LY-117018.[13] In this paper we focus on the effect of hydroxyl groups on agonist–antagonist profile of the DABP prototypes. This was of interest on account of the known propensity of OH groups, when at

Chart I



4,4′-position of the *trans*-stilbene core, to impart improved activity to TAE as well as TAP antiestrogens.[2-4,14] The

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⊥ Present address: Regional Research Laboratory, Jammu, India.

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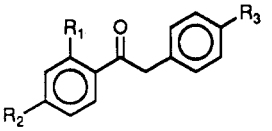
Chart II



2: R_1 = OH, R_2 = H, R_3 = OMe
3: R_1 = R_3 = OH, R_2 = H
4: R_1 = H, R_2 = OH, R_3 = OMe
5: R_1 = H, R_2 = R_3 = OH

implication of these and our earlier studies on SAR of DABPs[12,13] on molecular origins of estrogen agonist–antagonist activity are discussed.

The possible role of the recently discovered estrogen noncompetable antiestrogen specific binding site (AEBS) in modulating antitumor activity of antiestrogens[15-18] also prompted the evaluation of DABP phenols for their AEBS affinity. The results from this study also are presented.

Chemistry

Benzopyran **1** was synthesized according to the procedure reported earlier.[13] 2,3-Diaryl-2*H*-1-benzopyrans, carrying hydroxy or alkoxy substituents at para position in the aryl residues or at position 7 in the benzopyran nucleus, were synthesized via a previously described procedure involving condensation of appropriate desoxybenzoins with 4-hydroxybenzaldehyde.[12] The phenolic analogues bearing the piperidinoethoxy residue on 2-phenyl were prepared by starting from tetrahydropyranyl (THP) ethers of the appropriate desoxybenzoins, thus allowing chemical selectivity in attachment of the side chain to the requisite OH group.

The 2′-hydroxybenzoins **2** and **3**, prepared by Fries rearrangement of phenyl (4-methoxyphenyl)acetate, were obtained as a mixture along with the 4′-hydroxy isomers **4** and **5** (Chart II). The mixture was resolved chromatographically and an additional amount of dihydroxydesoxybenzoin **3** was obtained by demethylation of **2** with pyridine hydrochloride. The 2′-hydroxydesoxybenzoins **7** and **9** were prepared according to the reported procedures.[19,20] The phenolic desoxybenzoins **3** and **7** were next converted to the mono-THP ethers **6** and **8**, respectively, by reacting with dihydropyran using *p*-toluenesulfonic acid (PTSA) as a catalyst, while the trisphenol **9** was converted to the bis-THP ether **10** by using hydrochloric acid as a catalyst. The protected desoxybenzoins **6**, **8**, and **10** were next reacted with 4-hydroxybenzaldehyde in the presence of piperidine to furnish a mixture of corresponding dihydrobenzopyranones **11**, **13**, and **15** and 2-phenylchalcones **12**, **14**, and **16** (Scheme I). These were separated by rapid column chromatography and characterized on the

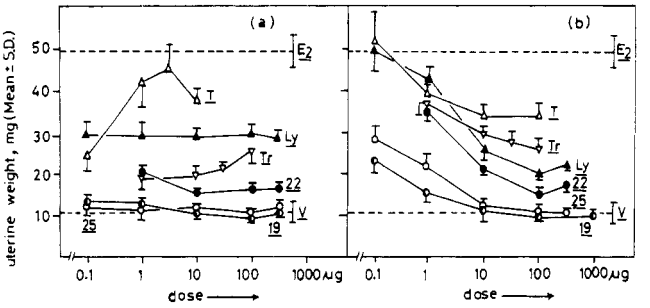
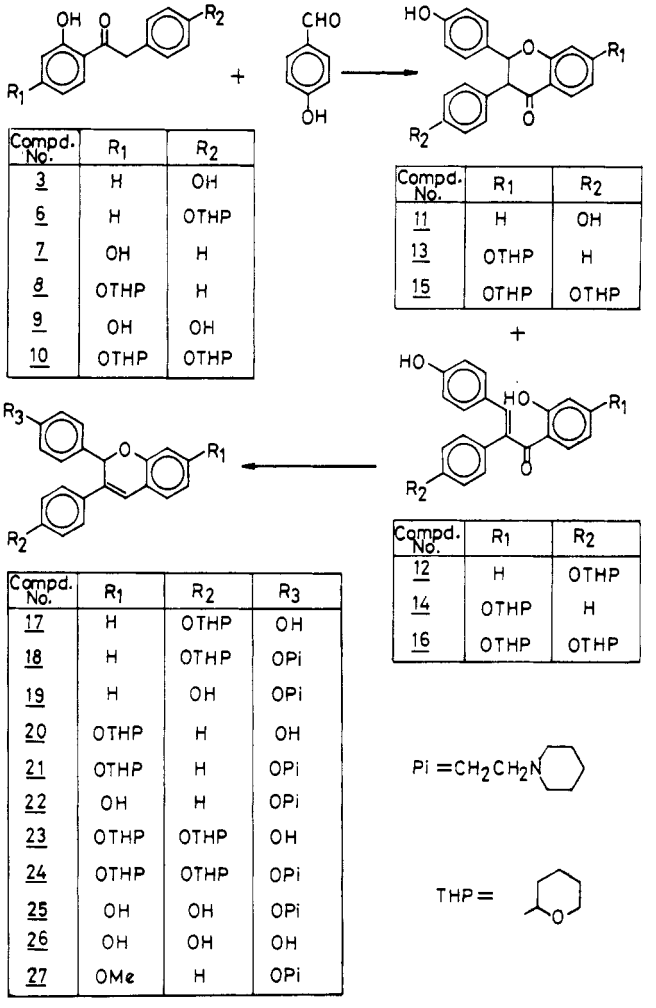


Figure 1. Uterotrophic (panel a) and antiuterophic (panel b) activities of hydroxybenzopyrans **19** (◐–◐), **22** (●–●), and **25** (○–○) and that of tamoxifen (T, △–△), trioxifen (Tr, ▽–▽), and LY-117018 (Ly, ▲–▲) at the indicated doses in immature female rats. Control group of animals received either the vehicle alone (V) or 0.3 μg of estradiol along with the vehicle (E_2). The values represent mean uterine weight ± SD in milligrams from six animals in each case.

Scheme I



Compd. No.	R_1	R_2
3	H	OH
6	H	OTHP
7	OH	H
8	OTHP	H
9	OH	OH
10	OTHP	OTHP

Compd. No.	R_1	R_2
11	H	OH
13	OTHP	H
15	OTHP	OTHP

Compd. No.	R_1	R_2
12	H	OTHP
14	OTHP	H
16	OTHP	OTHP

Compd. No.	R_1	R_2	R_3
17	H	OTHP	OH
18	H	OTHP	OPi
19	H	OH	OPi
20	OTHP	H	OH
21	OTHP	H	OPi
22	OH	H	OPi
23	OTHP	OTHP	OH
24	OTHP	OTHP	OPi
25	OH	OH	OPi
26	OH	OH	OH
27	OMe	H	OPi

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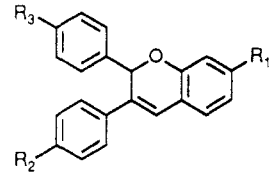
Table I. Receptor Affinity and Biological Activity Data of the Indicated Benzopyran Derivatives and That of the Tamoxifen, Trioxifen, annd LY-117018



compd	R_1	R_2	R_3	RBA-ER[a]	RBA-AEBS[b]	estrogenic activity[c]	antiestrogenic activity[c]	% inhibitor[d]
1	H	H	Pi[e]	0.3 ± 0.1	225 ± 26	15.3 ± 1.6	18.6 ± 1.4	75
19	H	OH	Pi	4.2 ± 0.2	14 ± 3	10.4 ± 1.0	11.6 ± 2.3	94
22	OH	H	Pi	9.5 ± 1.2	13 ± 6	15.2 ± 1.1	21.2 ± 2.0	65
25	OH	OH	Pi	49 ± 5	2.2 ± 0.9	10.6 ± 0.9	12.4 ± 1.3	98
26	OH	OH	OH	2.2 ± 0.2	-	14.2 ± 3.1	44.2 ± 1.3	0
27	OMe	H	Pi	0.02 ± 0.01	21 ± 3	22.6 ± 2.3	43.1 ± 5.2	0
		tamoxifen		1.8 ± 0.3	100	37.0 ± 1.7	34.4 ± 1.5	37
		trioxifen		8.0 ± 0.9	25 ± 7	18.6 ± 2.1	29.1 ± 1.7	40
		LY-117018		93 ± 5	6.5 ± 1.7	25.5 ± 1.2	25.6 ± 2.3	62

[a] The values are expressed as % of E_2 and are mean ± SD from at least three determinations. [b] The values are expressed as % of tamoxifen and are mean ± SD from at least three independent determinations. [c] A 10 μg/rat dose of each compound was administered on three consecutive days. The values represent mean uterine weight ± SD in milligrams from six to nine animals. For antiestrogenic activity, a 0.3 μg/animal dose of E_2 was coadministered. Control values are 45.1 ± 3.2 in the case of animals receiving 0.3 μg of E_2 plus vehicle and 11.5 ± 0.9 in the case of animals receiving the vehicle alone. [d] Computed as $(E - C_e)\ 100/(E - V)$; where in V, E, and C_e refer to the mean uterine weights from animals treated with vehicle alone, with E_2 alone, and with a given compound along with E_2, respectively. [e] Pi = piperidinoethoxy.

basis of their spectral and analytical data.

The 2-phenyl chalcones **12**, **14**, and **16** were next reduced with sodium borohydride in ethanol to afford the corresponding alcohols, which underwent smooth thermal cyclohydration in situ to afford the 2*H*-1-benzopyran phenols **17**, **20**, and **23**, respectively. The phenols were purified chromatographically and characterized on the basis of their spectral data. These phenols were next converted to the respective ethers **18**, **21**, and **24** by reaction with 2-piperidinoethyl chloride in the presence of potassium carbonate in acetone heated under reflux. The ethers were then subjected to the acid-catalyzed deprotection reaction to furnish the mono or dihydroxy benzopyran ethers **19**, **22**, and **25**.

The trisphenol **26** was prepared by deprotection of the bis-THP benzopyran phenol **23** by using hydrochloric acid as a catalyst, while the methoxybenzopyran ether **27** was obtained by reaction of 2-(4-hydroxyphenyl)-3-phenyl-7-methoxy-2*H*-1-benzopyran[12] with 2-piperidinoethyl chloride in the usual manner.

Results

The relative binding affinities (RBAs) of the test compounds for estrogen receptor (ER) from rat uterine cytosol and for AEBS from rat liver microsomal fraction were evaluated according to the procedures reported earlier.[9,21] The compounds were also assayed for their uterotrophic and antiuterotrophic activities in immature rats and mice. The results are shown in Table I and Figures 1 and 2.

ER and AEBS Affinities. The data on receptor affinity (Table I) reveal that all the compounds tested do interact with ER with their RBA values varying in a wide range. With RBA of 0.3 the benzopyran ether **1**, lacking in any phenolic group, is a modest receptor ligand. Receptor affinity of the molecule decreases further upon incorporation of a methoxy group at position 7 (compound **1** vs **27**), while incorporation of an OH at this position causes marked increase in its affinity (compound **1** vs **22**). Presence of an OH at para position in 3-phenyl also is beneficial for ER affinity of the molecule (compound **1** vs **19**). The dihydroxy analogue **25** is a more effective receptor ligand than both the monophenols **19** and **22**, and nearly hundred fold more potent than the benzopyran **1**. The basic ether chain has a marked contribution in receptor affinity of **25**, as its removal—to produce the trisphenol **26**—results in a substantial decrease in RBA of the molecule. ER affinities of tamoxifen, trioxifen, and LY 117018 were also evaluated, and the values (Table I) were found to be in close correspondence with those reported in literature.

All the compounds tested were found to act as AEBS ligands (Table I). The 2*H*-1-benzopyran **1** is the most potent AEBS ligand in the series, about twice as effective as tamoxifen. All the analogues carrying methoxy or hydroxy substituents, viz. **19**, **22**, and **27**, are much poorer AEBS ligands. The dihydroxybenzopyran **25** is the least effective AEBS ligand with RBA of about 2%. Trioxifen and LY-117017 are weaker AEBS ligand than tamoxifen. LY-117018 with two hydroxy groups is a poorer ligand than trioxifen featuring one methoxy.

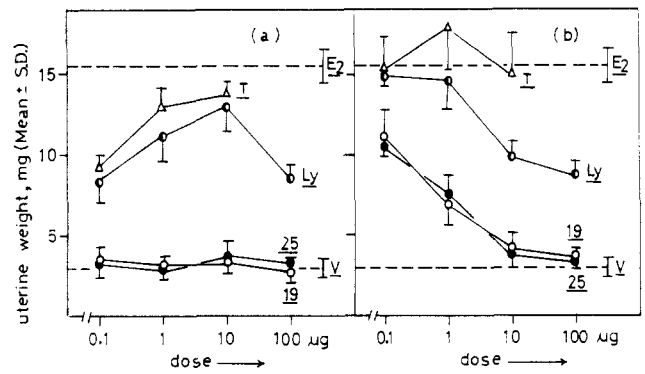


Figure 2. Uterotrophic (panel a) and antiuterotrophic (panel b) activities of hydroxybenzopyrans **19** (○–○) and **25** (●–●) and that of tamoxifen (T, Δ–Δ) and LY-117018 (◐–◐) at the indicated doses in immature female mice. Control group of animals received either the vehicle alone (V) or 0.1 μg of estradiol along with the vehicle (E_2). The values represent mean uterine weight ± SD in milligrams from at least seven animals in each case.

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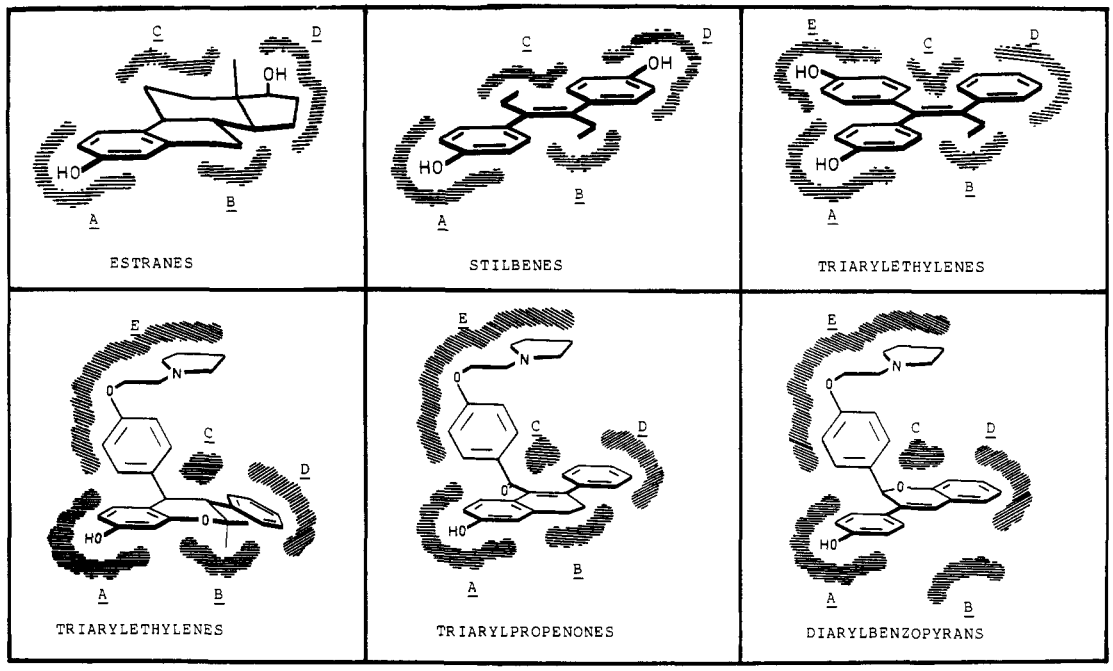

Figure 3. The model for binding site on estrogen receptor showing interaction of agonists (upper panels) and antagonists (lower panels) with the proposed subsites.

Estrogen Agonist and Antagonist Activities. From the data in Table I all compound except the trisphenol **26** are found to possess estrogen agonist or/and antagonist activities. The unsubstituted benzopyran **1** and the hydroxybenzopyran **22**, substituted at position 7, are both associated with weak uterotrophic activity and marked antiuterotrophic activity of comparable order. In relation to these the 7-methoxy analogue **27** has somewhat greater uterotrophic activity while being inactive as an antiestrogen. The benzopyran phenol **19** and the bisphenol **25** are both devoid of uterotrophic activity but act as very effective antiestrogens causing more than a 90% decrease in estradiol (E_2) stimulated uterine growth.

In view of their potent antagonist activities, the benzopyran phenols **19**, **22**, and **25** were taken up for detailed evaluation. Their uterotrophic and antiuterotrophic activities were evaluated in rats as well as mice, as a function of dose, and were compared with that of tamoxifen, trioxifen, and LY-117018. The results from this study in rats are presented in Figure 1. From the uterotrophic activity data (panel a) all the benzopyran phenols are found to be weaker agonists than the reference antiestrogens used. In accordance with the literature reports LY-117018 and trioxifen are weaker agonists than tamoxifen, but the benzopyran phenols are even weaker agonists than these. Among the benzopyrans, the monophenol **22** is found to be associated with a certain degree of agonist activity while the monophenol **19** and the diphenol **25** do not cause any significant increase in uterine weight. From the antiuterotrophic data (Figure 1, panel b) the benzopyrans are found to be more effective antagonists than the reference antiestrogens used. Tamoxifen, trioxifen, and LY-117018 and the benzopyran phenol **22** are all suboptimal antagonists with their activity following the order tamoxifen < trioxifen < LY-117018 ≤ **22**. The benzopyran phenols **19** and **25** are better antiestrogens than all these since they effect nearly complete inhibition of E_2 stimulated uterine growth.

The results of the comparative study of the benzopyran phenols **19** and **25**, tamoxifen, and LY-117018 in mice are shown in Figure 2. In this animal species as well the benzopyran phenols **19** and **25** show much greater antiuterotrophic activity than both the reference antiestrogens used. While tamoxifen as well as LY-117018 cause marked uterine growth, the benzopyran phenols do not produce any significant change in uterine weight even at the highest dose level (100 μg) employed. Tamoxifen is inactive in the antiuterotrophic assay while LY-117018 is only a suboptimal antiestrogen. The benzopyran phenols are equally effective antiestrogens and both are able to cause near complete inhibition of the E_2 stimulated growth at the higher (10 and 100 μg) dose levels.

Discussion

The marked antiestrogenic activity in benzopyran **1** together with its weaker agonist character than tamoxifen and LY-117018 was noticed earlier.[13] Incorporation of OH at 4′-position in 3 phenyl has now been found to further decrease agonist activity in **1** to almost imperceptible level. The DABP phenols **19** and **25** have thus emerged as potent antiestrogens devoid of any noticeable agonist activity in immature rats as well as mice. The hydroxyl group effects noticed here appear to be qualitatively the same as reported in literature for TAE and TAP antiestrogens.[2-4,14] We have earlier shown that DABPs also resemble other antiestrogens in their requirement of the side chain for biological activity.[13] From this it can be surmised that DABPs resemble TAEs and TAPs quite closely in their SAR. The receptor-binding model that we proposed earlier for TAE and related antiestrogens[8] seems in fact to provide the satisfactory framework for rationalization of SAR among TAP and DABP antiestrogens as well.

The 3D structures of a number of compounds representative of the major category of estrogen agonist and antagonists, reconstructed from their Dreiding models, are shown in Figure 3. The figure illustrates the possible interactions of the prototypes with the binding site which is visualized as a collection of five subsites A, B, C, D, and E. The enantiomers projected in the case of centchroman (panel 4) and the DABP prototype (panel 6) have been chosen arbitrarily. 4-Hydroxytamoxifen[14,22] devoid of the

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side chain (panel 3) has been included as the representative of the full agonists in the TAE category. Centchroman, being a nonplanar but conformationally constrained TAE antiestrogen, provides the reference for visualizing possible binding orientations in case of TAP (panel 5) and DABP (panel 6) prototypes.

The antagonist activity in TAE, TAP, and DABP prototypes reveals an obvious correlation with occupance of subsite E by the *p*-(tertiary-aminoalkoxy)aryl residue. Precise nature of the chain is known to influence the extent of antagonist potential in each antiestrogen. The pyrrolidinoethoxy and the piperidinoethoxy chains are the optimal structural requirements for the TAE antiestrogens[23-26] and the TAP and DABP antiestrogens,[6,13,27] respectively. The prototypes are further known to differ in the nature of response to deletion of the side chain. While such analogues in the TAE category usually are full agonists,[2,24,25] those in the TAP and DABP category are known to be devoid of agonist as well as antagonist activity.[12,27] While possible reasons for these differences remain unclear it is of note that the carbonyl in TAPs and the topologically equivalent C–O in DABPs, the feature that distinguishes them from the TAEs, constitute the part of substructure E in these molecules. The presence of the extra carbon also alters the spatial relationship of the alkoxyaryl residue and the stilbene core in these prototypes as compared to that in the TAEs. It is hence possible that TAPs and DABPs differ from TAEs in the precise nature of their interaction in subsite E.

The nature of interaction in subsite B appears to be the further factor influencing the agonist–antagonist balance in an antiestrogen. This view is supported by the correlation of greater agonist character of tamoxifen, trioxifen, and of a 4-ethyl-substituted DABP[13] with the presence in these molecules of an alkyl as substructure B and that of the diminished agonist character of LY-117018,[4,5] MER-25,[28,29] and of the DABPs such as **1** with the presence in these molecules of –S– or –H for substructure B.

The findings reported in this paper reveal further that the presence of an OH for interaction with subsite A can also be an important factor in antagonist efficacy. It is possible that this effect underlies the better antagonist activity in 4-hydroxytamoxifen than in tamoxifen[2,14] and in LY-117018 than in trioxifen.[4,5]

The agonist–antagonist profile of an antiestrogen thus seems to correlate with the nature of its subsite interaction. It is quite plausible that the subsite interaction dictates the conformational response that the ligand will evoke from the receptor protein which dictates in turn the extent and magnitude of activation of the receptor. The proposed binding model thus provides the framework that may explain the physical link between molecular architecture of a receptor ligand and its action as an agonist or antagonist.

A speculative model for conformational activation of estrogen receptor rationalizing the action of full agonists, partial agonists–antagonists, and full antagonists is illustrated in Figure 4. According to this model, the binding

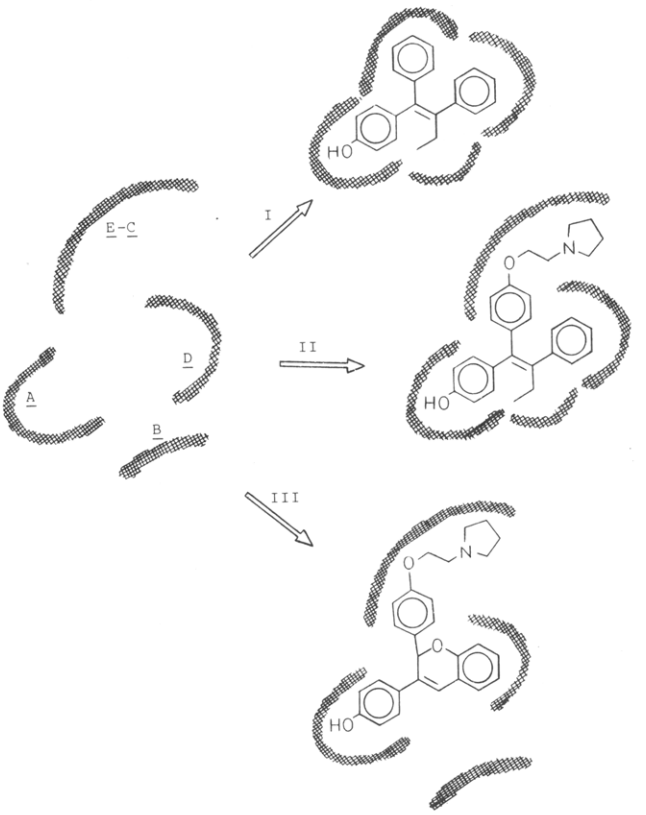

Figure 4. Conformational basis of receptor activation. The binding site in the native, unliganded receptor (left-hand side) showing the anchoring subsites A and D and the conformationally responsive subsites B and E–C, both of which respond on agonist interaction (I). In the interaction of partial antagonists (II) the subsite E–C does not respond, while in the interaction of full antagonists (III) neither subsite B nor E–C responds.

site in the native receptor is an open cleft capable of closure on an interacting agonist. The conformational change that accompanies the closure is the causative principle in the activation of the receptor. The initial interaction with all kinds of ligands is via subsites A and D, which constitute the anchoring subsites. This is followed, in case of agonists, by conformational changes in subsites B, C, and E, resulting in the closure of the binding site. Presence of an appropriately placed hydroxyl facilities this process by providing better anchorage to the ligand in subsite A. Although TAE agonists lack the substructure C while other agonists, such as estradiol, lack the substructure E, the overall conformation of their respective receptor complexes is possibly the same. This may be because of the conformational coupling between subsites E and C which may be the adjoining regions of a common domain.

In the case of TAE antiestrogens the initial interaction with subsites A and D is followed by the normal conformative response in subsite B while the composite subsite E–C does not respond due to its interaction with the side chain. The resultant complex is in the state of partial activation. In the case of DABP antiestrogens the conformational change in subsite E–C is prevented due to the presence of the side chain while conformational change in subsite B does not occur due to the absence of a residue to favorably stabilize it. The resultant complex is functionally inert because its conformation is closer to that of the native protein.

AEBS affinity of benzopyran **1** decreases substantially on incorporation of OH and methoxy groups into the molecule. The decrease correlates with the number of such groups incorporated. The results further substantiate that

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AEBS affinity has no correlation with antiestrogenic potency of the prototypes. However in view of their AEBS affinity the prototypes may be useful aids in establishing whether or not these sites are involved in antitumor activity of antiestrogens.

Experimental Section

The melting points were taken on a Towson and Mercer's (U.K.) melting point apparatus and are uncorrected. The IR spectra (V_{max}) were recorded on Perkin-Elmer 157 or 177 instruments as KBr wafers or as neat films and values are reported in the cm^{-1} scale. The ^1H NMR spectra were recorded either on Varian CFT-20, on Perkin-Elmer R-32, or on Varian EM-360 spectrometers, with tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as the solvent unless indicated otherwise. The values are given in the δ scale. The mass spectra were run on a JEOL-JMS D 300 instrument. The homogeneity of compounds was routinely checked on silica gel or neutral alumina plates.

The synthesis of benzopyran **1** has been reported earlier.[13]

1-(2-Hydroxyphenyl)-2-(4-hydroxy(or methoxy)phenyl)-ethanone (2 and 3) and 1-(4-Hydroxyphenyl)-2-(4-hydroxy(or methoxy)phenyl)ethanone (4 and 5). A mixture of phenyl (4-methoxyphenyl)acetate (121 g) and anhydrous aluminum chloride (93 g) was heated for 1.5 h at 145 °C. It was then cooled, treated with a mixture of ice (500 g) and concentrated hydrochloric acid and then was extracted with EtOAc (2 × 300 mL). The organic layer was washed with water (2 × 100 mL), dried (Na_2SO_4) and concentrated. The residue was allowed to stand for 12 h to solidify. The solid material was filtered off and washed with chloroform to afford **5** (35 g), mp 212 °C (lit.[30] mp 215 °C). The combined filterate was then concentrated, and the residue was chromatographed over a column of silica gel with EtOAc–hexane (1:30, v/v) as eluant to afford **2** (15.5 g) which was crystallized from chloroform–hexane: mp 86 °C; IR 1640 (CO); ^1H NMR 3.70 (s, 3 H, OCH_3), 4.10 (s, 2 H, $-COCH_2-$), 6.70–6.90 (m, 4 H, Ar-*H*, *o* to OH and *o* to OCH_3), 7.10 (d, 2 H, J = 8.0 Hz, Ar-*H*, *m* to OCH_3), 7.30 (t, 1 H, J = 8.0 Hz, Ar-*H*, *p* to CO), 7.70 (dd, 1 H, J = 8.0 and 2.0 Hz, Ar-*H*, *o* to CO), 13.00 (s, 1 H, O*H*); MS m/z 242 (M^+). Anal. ($C_{15}H_{14}O_3$) C and H. Further elution with EtOAc–hexane (1:20, v/v) afforded first **3** (14 g), mp 140 °C (lit.[20] mp 106 °C), and then **4** (15 g), mp 175 °C (lit.[31] mp 175 °C), which were crystallized from EtOAc–hexane.

Conversion of 2 into 3. A mixture of the methoxyhydroxydesoxybenzoin **2** (12.1 g) and anhydrous pyridine hydrochloride (30 g) was heated at 220 °C for 1 h. It was cooled, treated with water (100 mL), and extracted with EtOAc (2 × 200 mL). The organic layer was washed with water (2 × 50 mL), dried (Na_2SO_4), and concentrated. The residue was filtered through a short column of silica gel with EtOAc–hexane (1:10, v/v) as eluant to afford **3** (9 g) which was crystallized from EtOAc–hexane.

1-(2-Hydroxyphenyl)-2-[4-[(tetrahydropyran-2-yl)oxy]-phenyl]ethanone (6). To a stirred solution of the deoxybenzoin **3** (18.3 g) and PTSA (100 mg) in dry dioxane (200 mL) was added dropwise a solution of 3,4-dihydro-2*H*-pyran (14.5 mL) in dry dioxane (100 mL) and the stirring was continued for 4 h. The reaction was then neutralized with methanolic ammonia (5 mL) and concentrated in vacuo. The residue was dissolved in ether (200 mL) and washed with 5% sodium hydroxide solution (2 × 50 mL), followed by water (2 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was chromatographed over a short column of silica gel by rapidly eluting with EtOAc–hexane (1:20, v/v), to afford **6** (20 g) which was crystallized from hexane: mp 95 °C; IR 1640 (CO); ^1H NMR (CCl_4) 1.40–1.80 (m, 6 H, 3′-, 4′-, 5′-*H*), 3.40–3.80 (m, 2 H, 6′-*H*), 4.00 (s, 2 H, $COCH_2$), 5.20 (br s, 1 H, 2′-*H*), 6.50–6.90 (m, 4 H, Ar-*H*, *o* to (tetrahydropyranyl)oxy and *o* and *p* to OH), 7.00 (d, 2 H, J = 8.0 Hz, Ar-*H*, *m* to OH), 7.20 (t, 1 H, J = 8.0 Hz, Ar-*H*, *p* to CO), 7.60 (dd, 1 H, J = 8.0 and 2.0 Hz, Ar-*H*, *o* to CO), 14.00 (s, 1 H, O*H*, *o* to CO); MS m/z 227 (M^+). Anal. ($C_{19}H_{20}O_4$) C and H.

1-[2-Hydroxy-4-[(tetrahydropyran-2-yl)oxy]phenyl]-2-phenylethanone (8). To a stirred solution of the 1-(2,4-dihydroxyphenyl)-2-phenylethanone[19] (**7**, 8.3 g) and PTSA (100 mg) in dry dioxane (200 mL) was added a solution of 3,4-dihydro-2*H*-pyran (14.5 mL) in dry dioxane (200 mL), and the stirring was continued for 5 h. The reaction mixture was worked up by using the procedure as described for **6** to afford a residue which was chromatographed over a column of silica gel by rapidly eluting with EtOAc–hexane (1:20, v/v) to furnish **8** as an oil (20 g). The product was crystallized from hexane: mp 89 °C; IR 1620 (CO); ^1H NMR 1.50–1.90 (m, 6 H, 3′-, 4′-, and 5′-*H*), 3.50–3.90 (m, 2 H, Ar-*H*, *o* to (tetrahydropyranyl)oxy), 7.20 (s, 5 H, Ar-*H*), 7.80 (d, 1 H, J = 8.0 Hz, Ar-*H*, *o* to CO); MS m/z 228 ($M^+ - 84$). Anal. ($C_{19}H_{20}O_4$) C and H.

1-[2-Hydroxy-4-[(tetrahydropyran-2-yl)oxy]phenyl]-2-[4-[(tetrahydropyran-2-yl)oxy]phenyl]ethanone (10). To the 1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)ethanone[20] (**9**, 2.44 g) cooled in an ice bath was added a mixture of 3,4-dihydro-2*H*-pyran (10 mL) and concentrated hydrochloric acid (0.01 mL), and the reaction mixture was stirred for 4 h. It was then diluted with ether (100 mL) and washed with 5% sodium hydroxide solution (3 × 25 mL), followed by water (2 × 25 mL). The ether layer was dried (Na_2SO_4) and concentrated. The oily residue was crystallized from hexane to furnish **10** (3.6 g): mp 118 °C; IR 1640 (CO); ^1H NMR (CCl_4) 1.30–1.90 (m, 12 H, 3′-, 4′-, and 5′-*H*), 3.20–3.80 (m, 4 H, 6′-*H*), 3.90 (s, 2 H, $COCH_2$), 5.20–5.40 (each br s, 2 H, 2 × 2′-*H*), 6.20–6.40 (m, 2 H, Ar-*H*, *o* to (tetrahydropyranyl)oxy), 6.80 (d, 2 H, J = 8.0 Hz, Ar-*H*, *o* to (tetrahydropyranyl)oxy), 7.00 (d, 2 H, J = 8.0 Hz, Ar-*H*, *m* to (tetrahydropyranyl)oxy), 7.50 (d, 1 H, J = 8.0 Hz, Ar-*H*, *o* to CO) and 13.0 (s, 1 H, O*H*); MS m/z 327 ($M^+ - 85$). Anal. ($C_{24}H_{28}O_6$) C and H.

2,3-Bis(hydroxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (11) and 1-(2-Hydroxyphenyl)-2-[4-[(tetrahydropyran-2-yl)oxy]phenyl]-3-(4-hydroxyphenyl)pro-2-en-1-one (12). To a solution of desoxybenzoin **6** (6.9 g) and 4-hydroxybenzaldehyde (2.5 g) in dry benzene (100 mL) was added dry piperidine (0.12 mL). The mixture was heated under reflux for 30 h while water was removed azeotropically. It was then cooled and washed with water (2 × 40 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was chromatographed over a column of silica gel by rapidly eluting with EtOAc–hexane (1:20, v/v) to afford first the unreacted desoxybenzoin **6** and then, on increasing the solvent polarity (1:5, v/v), the phenylchalcone **12** (6 g) which was crystallized from benzene–hexane: mp 140 °C; IR 3300 (OH), 1620 (CO); ^1H NMR 1.40–1.90 (m, 6 H, 3′-, 4′-, and 5′-*H*), 3.40–3.80 (m, 2 H, 6′-*H*), 5.40 (br s, 1 H, 2′-*H*), 6.30 (s, 1 H, exchangeable H^+), 6.80–7.80 (m, 13 H, Ar-*H* and olefinic *H*), 14.0 (s, 1 H, O*H*, *o* to CO); MS m/z 416 (M^+), 331 ($M^+ - 85$). Anal. ($C_{26}H_{24}O_5$) C and H.

Further elution afforded the dihydrobenzopyranone **11** (1 g) which was crystallized from EtOAc: mp 260 °C; IR 3350 (OH), 1680 (CO); ^1H NMR (DMSO-d_6 + $CDCl_3$) 4.15 (d, 1 H, J = 12.0 Hz, $-COCH-$), 5.50 (d, 1 H, J = 12.0 Hz, $-OCH-$), 6.40–7.20 (m, 10 H, Ar-*H*), 7.40 (td, 1 H, J = 8.0 and 2.0 Hz, Ar-*H*, *p* to CO), 7.75 (dd, 1 H, J = 8.0 and 2.0 Hz, Ar-*H*, *o* to CO); MS m/z 332 (M^+). Anal. ($C_{21}H_{16}O_4$) C and H.

2-(4-Hydroxyphenyl)-3-phenyl-7-[(tetrahydropyran-2-yl)oxy]-2,3-dihydro-4*H*-1-benzopyran-4-one (13) and 1-[2-Hydroxy-4-[(tetrahydropyran-2-yl)oxy]phenyl]-2-phenyl-3-[4-hydroxyphenyl]prop-2-en-1-one (14). These were obtained in about 30% overall yield by heating under reflux a mixture of desoxybenzoin **8** (6.9 g) and 4-hydroxybenzaldehyde (2.4 g) in dry benzene (100 mL) in presence of dry piperidine (0.12 mL), followed by the workup and purification procedure as described for **11** and **12**. **13** (crystallized from EtOAc–hexane): mp 180 °C; IR 3450 (OH), 1680 (CO); ^1H NMR (acetone-d_6) 1.50–1.90 (m, 6 H, 3′-, 4′-, and 5′-*H*), 3.40–3.80 (m, 2 H, 6′-*H*), 4.20 (d, 1 H, J = 12.0 Hz, $-COCH-$), 5.45 (br s, 1 H, 2′-*H*), 5.55 (d, 1 H, J = 12.0 Hz, $-OCH-$), 6.50–6.70 (m, 4 H, Ar-*H*, *o* to (tetrahydropyranyl)oxy and OH), 6.95–7.15 (m, 7 H, Ar-*H*), 7.70 (d, 1 H, J = 8.0 Hz, Ar-*H*, *o* to CO); MS m/z 416 (M^+), 331 ($M^+ - 85$). Anal. ($C_{26}H_{24}O_5$) C and H. **14** (2.5 g), obtained as an oil, was used in the next step without crystallization and spectral characterization.

2-(4-Hydroxyphenyl)-3-[4-[(tetrahydropyran-2-yl)oxy]-phenyl]-7-[(tetrahydropyran-2-yl)oxy]-2,3-dihydro-4*H*-1-

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benzopyran-4-one (15) and 1-[2-Hydroxy-4-[(tetrahydropyran-2-yl)oxy]phenyl]-2-[4-[(tetrahydropyran-2-yl)oxy]phenyl]-3-(hydroxyphenyl)prop-2-en-1-one (16). These were obtained by heating under reflux a mixture of desoxybenzoin **10** (4.12 g) and 4-hydroxybenzaldehyde (1.22 g) in dry benzene (100 mL) and dry piperidine (0.01 mL) followed by workup and purification procedure as detailed for **11** and **12**. **15** (1.6 g) crystallized from EtOAc–hexane: mp 190 °C; IR 3400 (OH), 1680 (CO); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 1.30–1.80 (m, 12 H, 3′-, 4′-, and 5′-*H*), 3.30–3.80 (m, 4 H, 6′-*H*), 4.00 (d, 1 H, J = 12.0 Hz, –COC*H*–), 5.10–5.50 (m, 3 H, –OC*H*– and 2′-*H*), 6.40–7.10 (m, 10 H, Ar-*H*), 7.70 (d, 1 H, J = 8.0 Hz, Ar-*H*, *o* to CO); MS m/z 431 (M^+ – 85). Anal. ($C_{31}H_{32}O_7$) C and H. **16** (1.5 g), obtained as an oil, was used in the next step without crystallization and spectral characterization.

2-(4-Hydroxyphenyl)-3-[4-[(tetrahydropyran-2-yl)oxy]phenyl]-2*H*-1-benzopyran (17). To a stirred solution of the 2-phenylchalcone **12** (4.2 g) in ethanol (50 mL) was added sodium borohydride (400 mg), and the stirring was continued for 15 h. Ethanol was evaporated in vacuo and to the residue was added saturated ammonium chloride solution dropwise until the pH was 8.0. The mixture was extracted with EtOAc (2 × 60 mL), and the organic layer was washed with water (2 × 10 mL), dried (Na_2SO_4), and then concentrated. The residue was flash chromatographed over a silica gel column, with EtOAc–hexane (1:10, v/v) as eluant, to afford **17** (2.4 g), which was crystallized from benzene–hexane: mp 110 °C; IR 3350 (OH); ^{1}H NMR 1.30–1.80 (m, 6 H, 3′-, 4′-, and 5′-*H*), 3.30–3.90 (m, 2 H, 6′-*H*), 5.30 (br s, 2 H, 2′-*H* and exchangeable H^+), 6.00 (s, 1 H, –OC*H*–), 6.40–7.30 (m, 13 H, Ar-*H*); MS m/z 400 (M^+). Anal. ($C_{26}H_{24}O_4$) C and H.

2-(4-Hydroxyphenyl)-3-phenyl-7-[(tetrahydropyran-2-yl)oxy]-2*H*-1-benzopyran (20). To a stirred solution of the 2-phenylchalcone **14** (1.25 g) in ethanol (10 mL) was added sodium borohydride (120 mg), and the stirring was continued for 12 h. The workup of the reaction mixture, using the procedure as described for **17**, afforded a residue which on flash chromatography over a silica-gel column, eluting with EtOAc–hexane (1:10, v/v), afforded **20** (600 mg), which was crystallized from chloroform–hexane, mp 154 °C; IR 3350 (OH); ^{1}H NMR 1.40–1.90 (m, 6 H, 3′-, 4′-, and 5′-*H*), 3.50–3.90 (m, 2 H, 6′-*H*), 5.35 (br s, 1 H, 2′-*H*), 6.10 (s, 1 H, –OC*H*–), 6.40–6.70 (m, 9 H, Ar-*H* and olefinic *H*); MS m/z 400 (M^+), 315 (M^+ – 85). Anal. ($C_{26}H_{24}O_4$) C and H.

2-(4-Hydroxyphenyl)-3-[4-[(tetrahydropyran-2-yl)oxy]phenyl]-7-[(tetrahydropyran-2-yl)oxy]-2*H*-1-benzopyran (23). To a stirred solution of the 2-phenylchalcone **16** (1.3 g) in ethanol (20 mL) was added sodium borohydride (100 mg) and the stirring continued for 12 h. The workup of the reaction mixture as described for **17** afforded a residue which was flash chromatographed over a column of silica gel with EtOAc–hexane (1:6, v/v) as eluant to furnish **23** (75 mg). It was crystallized from chloroform–hexane: mp 180 °C; IR 3400 (OH); ^{1}H NMR (acetone-d_6) 1.30–1.80 (m, 12 H, 3′-, 4′-, and 5′-*H*), 3.20–3.90 (m, 4 H, 6′-*H*), 5.20 and 5.30 (each br s, 2 H, 2 × 2′-*H*), 6.10 (s, 1 H, –OC*H*–), 6.20–7.30 (m, Ar-*H*); MS m/z 415 (M^+ – 85). Anal. ($C_{31}H_{32}O_6$) C and H.

General Procedure for Etherification and Deprotection of Tetrahydropyranyl Ethers. The following general procedure was used for converting the benzopyran phenols **17**, **20**, **23**, and 2-(4-hydroxyphenyl)-3-phenyl-7-methoxy-2*H*-1-benzopyran[12] into the basic ethers **18**, **21**, **24**, and **27**. A mixture of the phenol (2 mmol), anhydrous potassium carbonate (3.2 mmol), and dry acetone (30 mL) was heated under reflux with stirring for 30 h. After the reaction mixture was cooled, the solid material was filtered and washed with acetone. The combined filterate was concentrated and the residue was chromatographed over a basic alumina column, with EtOAc–hexane (1:50, v/v) as eluant, to afford the requisite basic ethers as oil, invariably in about 80% yield, which were directly submitted to the deprotection reaction.

The following general procedure was used for deprotection of the THP ethers **18**, **21**, **23**, and **24**. For each tetrahydropyran groups, HCl (5 mL) was added to a solution of the pyranyl ether (2 mmol) in ethanol (20 mL). The solution was allowed to stand for 20 min at room temperature and then was concentrated in vacuo. The residue after treatment with saturated sodium bicarbonate solution (20 mL) was extracted with EtOAc (2 × 50 mL). The organic layer was washed with water (2 × 20 mL), dried (Na_2SO_4), and concentrated to furnish the hydroxy compound as an oil in about 60% yield. These were crystallized as such or as oxalate salts. The data of the compounds thus prepared are given below:

2-[4-(2-Piperidinoethoxy)phenyl]-3-(4-hydroxyphenyl)-2*H*-1-benzopyran (19). Crystallized from chloroform–hexane: mp 110 °C; IR 3400–3200 (OH); ^{1}H NMR 1.20–1.70 (m, 6 H, $-CH_2(CH_2)_3CH_2-$), 2.35–2.60 (m, 4 H, $-CH_2NCH_2-$), 2.70 (t, 2 H, J = 6.0 Hz, $-OCH_2CH_2-$), 3.90 (t, 2 H, J = 6.0 Hz, $-OCH_2CH_2-$), 6.05 (s, 1 H, –OC*H*–), 6.40–7.30 (m, 13 H, Ar-*H* and olefinic *H*); MS m/z 427 (M^+). Anal. ($C_{28}H_{29}NO_3N$) C, H, and N.

2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-7-hydroxy-2*H*-1-benzopyran (22). Crystallized from absolute ethanol: mp 224 °C; IR 3400–3100 (OH); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 1.20–1.70 (m, 6 H, $-CH_2(CH_2)_3CH_2-$), 2.20–2.45 (m, 4 H, $-CH_2NCH_2-$), 2.55 (t, 2 H, J = 6.0 Hz, $-OCH_2CH_2-$), 3.90 (t, 2 H, J = 6.0 Hz, $-OCH_2CH_2-$), 6.00–6.40 (m, 3 H, OC*H* and Ar-*H*, *o* to OH), 6.80 (d, 2 H, J = 8.0 Hz, Ar-*H*, *o* to $-OCH_2-$), 6.90–7.50 (m, 9 H, Ar-*H* and olefinic *H*); MS m/z 427 (M^+). Anal. ($C_{28}H_{29}O_3N$) C, H, and N.

2-[4-(2-Piperidinoethoxy)phenyl]-7-hydroxy-2*H*-1-benzopyran (25). Crystallized as its oxalate salt from absolute ethanol–dry ether: mp 190 °C; IR (neat) 3400–3200 (OH); ^{1}H NMR (acetone-d_6) 1.30–1.70 (m, 6 H, $-CH_2(CH_2)_3CH_2-$), 2.20–2.45 (m, 4 H, $-CH_2NCH_2-$), 2.55 (t, 2 H, J = 6.0 Hz, $-OCH_2CH_2-$), 3.90 (t, 2 H, J = 6.0 H_2, $-OCH_2CH_2-$), 5.85 (br s, 2 H, exchangeable H^+), 6.00–6.30 (m, 3 H, –OC*H*– and Ar-*H*, *o* to OC), 6.60 (d, 4 H, J = 8.0 Hz, Ar-*H*, *o* to OH and $-OCH_2CH_2-$), 6.80–6.90 (m, 2 H, Ar-*H* and olefinic *H*), 7.20 (dd, 4 H, J = 8.0 and 2.0 Hz, Ar-*H*, *m* to OH and $-OCH_2CH_2-$); MS m/z 443 (M^+). Anal. ($C_{28}H_{29}O_4N{\cdot}CO_2H{\cdot}H_2O$) C, H, and N.

2,3-Bis(4-hydroxyphenyl)-7-hydroxy-2*H*-1-benzopyran (26). Crystallized from EtOAc–hexane: mp 120 °C; IR 3300 (OH); ^{1}H NMR (acetone-d_6) 6.10 (br s, 2 H, –OC*H*– and Ar-*H*, *o* to OH), 6.30 (d, 1 H, J = 8.0 Hz, Ar-*H*, *o* to OH), 6.65 and 6.70 (each d, 4 H, J = 8.0 Hz, 2 × Ar-*H*, *o* to OH), 6.95–7.05 (m, 2 H, Ar-*H* and olefinic *H*), 7.25 and 7.30 (each d, 4 H, J = 8.0 Hz, 2 × Ar-*H*, *o* to OH); MS m/z 332 (M^+). Anal. ($C_{21}H_{16}O_4$) C and H.

2-[4-(2-Piperidinoethoxy)phenyl]-3-phenyl-7-methoxy-2*H*-1-benzopyran (27). Crystallized from absolute ethanol–dry ether as oxalate salt: mp 134 °C; ^{1}H NMR (CCl_4) 1.30–1.70 (m, 6 H, $-CH_2(CH_2)_3CH_2-$), 2.25–2.45 (m, 4 H, $-CH_2NCH_2-$), 2.55 (t, 2 H, J = 6.0 Hz, $-OCH_2CH_2-$), 3.60 (s, 3 H, OCH_3), 3.85 (t, 2 H, J = 6.0 Hz, OCH_2CH_2), 6.00 (s, 1 H, –OC*H*–), 6.10–6.35 (m, 2 H, Ar-*H*, *o* to OCH_3), 6.60 (d, 2 H, J = 8.0 Hz, Ar-*H*, *o* to $-OCH_2CH_2-$), 6.80–7.30 (m, 11 H, Ar-*H* and olefinic *H*); MS m/z 441 (M^+). Anal. ($C_{29}H_{31}O_3N{\cdot}CO_2H{\cdot}H_2O$) C, H, and N.

Biology. Materials. [2,3,6,7-^3H]Estradiol (^{3}H-E_2, 100 Ci mmol^{-1}) and [N-Methyl-^3H]tamoxifen (^{3}H-Tam, 76 Ci mmol^{-1}) were purchased from New England Nuclear Corp. and were assessed as 95% radiochemically pure by use of Panax radio TLC scanner. Unlabeled estradiol (E_2) was obtained from Steraloids Inc., activated charcoal, Norit A, from Sigma Chemicals, and Dextran T-70, from Pharmacia Fine Chemicals. All other chemicals and reagents were of analytical or scintillation grade. Female mice (21–23 days old; 8–12 g body weight) and rats (21–23 days old; 25–40 g body weight) of Swiss and Charles Foster strain, respectively, were obtained from the C.D.R.I. rodent colony. For ER binding experiments the rats were primed subcutaneously with 0.1 μg of E_2 each, 24 h prior to sacrifice, to increase yield of the receptor protein in their uteri.

Competition Experiments. ER binding experiments were a minor modifications of the previously reported procedure.[9] Briefly, 50-μL aliquots of cystosol (one uterine equivalent per milliliter of TEA buffer) were incubated at 4 °C for 18–20 h with increasing concentrations of the test compounds (10^{-4}–10^{-9} M), in triplicate, and fixed concentration of ^{3}H-E_2 (5×10^{-9} M). Each incubate (70 μL) in TEA buffer (Tris-HCl, 10 mmol; ethylenediaminetetraacetic acid (EDTA), 165 mmol; NaN_3, 0.2%, pH 7.4) was 7% in dimethylformamide (DMF). Free and bound ^{3}H-E_2 were separated by treating each incubate with 10 μL of charcoal–dextran slurry (2.5 and 0.25% w/v, respectively) in TEA buffer for 20 min. Radioactivity of a 50-μL aliquot of each incubate was measured in minivials containing 5 mL of scintillation fluid (1.5:2.5:2.5, v/v mixture of methanol–dioxane–toluene containing 0.5% 2,5-diphenyloxazole (PPO), 0.01% [1,4-bis(5-

phenyloxazol-2-yl)benzene] (POPOP).

The competition experiments with liver microsomal fraction, using ^{3}H-Tam as the reference ligand, were performed according to the previously reported procedure.[21] The liver microsomal fractions were first incubated at 4 °C for 2 h, with 2 μM diethylstibestrol added in a small volume of DMF to saturate ER sites. Aliquots (200 μL) of the fraction were then mixed in a Pyrex glass tube with 20 μL of competitor (1×10^{-9} M to 3×10^{-6} M) and 20 μL of ^{3}H-Tam (1×10^{-9} M) dissolved in 35% DMF–TEA buffer. The tubes were incubated for 18 h at 4 °C and then treated with charcoal–dextran slurry (100 μL) for 15 min at 4 °C to separate bound and free ^{3}H-Tam. The tubes were centrifuged at 1000*g* for 15 min, and the supernatants were counted for radio activity.

Uterotrophic and Antiuterotrophic Activity. For uterotrophic activity various doses of the test compounds, suspended in 0.1 mL of propylene glycol–0.9% saline (1:1, v/v), were injected subcutaneously to the test animals on three consecutive days, while the control group of animals received the vehicle alone. Antiuterotrophic assay was similarly performed by administering various doses of test compounds and 0.3 μg of E_2 in the case of rats and 0.1 μg of E_2 in the case of mice, each suspended in 0.1 mL of propylene glycol–0.9% saline (1:1, v/v), to the test animals at two different sites, while the control group of animals received the injection of E_2 and the vehicle alone. Animals were autopsied 24 h after last injection and their uterine wet weights were recorded in the usual manner.

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Additions and Corrections

Volume 33, 1990

Manfred Reiffen,* Wolfgang Eberlein, Peter Müller, Manfred Psiorz, Klaus Noll, Joachim Heider, Christian Lillie, Walter Kobinger, and Peter Luger: Specific Bradycardic Agents. 1. Chemistry, Pharmacology, and Structure–Activity Relationships of Substituted Benzazepinones, a New Class of Compounds Exerting Antiischemic Properties.

Page 1496. The correct contribution line should read as follows: Department of Chemical Research, Dr. Karl Thomae GmbH, Postfach 1755, D-7950 Biberach 1, West Germany, Department of Pharmacology, Ernst-Boehringer-Institut für Arzneimittelforschung, Dr. Boehringer-Gasse 5-11, A-1121 Wien, Austria, and Freie Universität Berlin, Institut für Kristallographie, Takustra ıse 6, 1000 Berlin 33, West Germany.

P. S. Portoghese,* M. Sultana, and A. E. Takemori: Design of Peptidomimetic δ Opioid Receptor Antagonists Using the Message–Address Concept.

Page 1714. In Table III, the δ K_i (SE) value for compound **20** (OMI) should read 1.5 (0.4–5.1).