Synthesis of 2-(p-Chlorobenzyl)-3-aryl-6-methoxybenzofurans as Selective Ligands for Antiestrogen-Binding Sites. Effects on Cell Proliferation and Cholesterol Synthesis

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A series of nonsteroidal compounds, 2-(p-chlorobenzyl)-3-aryl-6-methoxybenzofurans derived from the 2-(p-chlorobenzyl)-6-methoxy-3(2H)-benzofuranones has been synthesized. The key steps in the synthesis were reactions of 2-(p-chlorobenzyl)-6-methoxy-3(2H)-benzofuranones with the arylorganometallic reagents followed by dehydration of the resulting carbinols. The benzofurans are ligands for antiestrogen-binding sites (AEBS) and display no significant interaction with the estrogen receptor (ER). All bind to AEBS with equivalent or greater affinity than tamoxifen. These compounds decrease [^{3}H]thymidine incorporation in AEBS-containing EL4 lymphoid cells and MCF7 breast cancer cells in a concentration-dependent manner between 10^{-8} and 10^{-6} M and are generally more inhibitory than tamoxifen. In contrast, they have no effect on [^{3}H]thymidine incorporation by an AEBS-deficient variant of the MCF7 cell line, RTx6. The present findings of (1) selective and high affinity binding of the benzofurans to AEBS, (2) their concentration-dependent inhibition of [^{3}H]thymidine incorporation in AEBS-containing cells, and (3) their lack of antiproliferative effect in an AEBS-deficient cell line suggest a functional role for AEBS in mediating the antigrowth effect of these compounds. Two of the more active benzofuran compounds also significantly inhibited de novo cholesterol biosynthesis in EL4 cells which lack ER. This effect could be obtained after 5 h of treatment and preceded significant loss of cell viability. This is the first demonstration that selective ligands of AEBS (other than the known nonsteroidal antiestrogens) interfere with cholesterol biosynthesis—an action that may contribute to their antigrowth effect.

Introduction

Nonsteroidal antiestrogens such as tamoxifen (1a) (Chart I) are established therapeutic agents for estrogen-dependent tumors, principally breast cancer. There is much indirect but persuasive evidence that antiestrogens act through a receptor-mediated mechanism in estrogen receptor-containing cell types.[1] The pharmacobiology of this class of drugs, however, is complex. In particular, the ability of antiestrogens to inhibit the growth of estrogen receptor-negative cells lacks an adequate explanation.[2]

Antiestrogens bind to two distinct intracellular proteins viz. the estrogen receptor (ER) and antiestrogen-binding sites (AEBS). AEBS differ from the estrogen receptor in ligand-binding specificity, physical characteristics, tissue and subcellular distribution.[3-6] Whether AEBS have any role in mediating nonreceptor-dependent inhibition of cellular proliferation is unresolved. Several observations are inconsistent with such a function. Growth responses of tumor cell lines were reported to be independent of AEBS levels.[7,8] A selective but low affinity ligand for AEBS failed to inhibit cell proliferation on its own and was inactive as a biological antagonist of estrogen action.[9]

However, data from other studies suggest that AEBS may mediate the receptor-independent antiproliferative effect of antiestrogens. The first clear evidence came from studies of an AEBS-deficient cell line, RTx6, which had an antiestrogen-resistant phenotype. The fact that RTx6 had ER of equivalent affinity and concentration as its antiestrogen-sensitive parental cell line, MCF7, appeared to implicate AEBS in mediating the antigrowth effect of antiestrogens.[10] More direct evidence came from observations that antiestrogens had dose-dependent cytostatic and cytotoxic effects in lymphoid cell lines lacking ER but rich in AEBS.[11,12] These findings do not, of course, establish the involvement of AEBS in the mechanism of action of antiestrogens. Several studies have attempted to address this question by examining a large number of compounds (different chemical classes of nonsteroidal antiestrogens, diphenylmethane derivatives,[13] chromenes,[14] oxygenated sterols,[15] and other compounds[16] such as phenothiazine, thioxanthine, stilbene, and bibenzyl de-

[†] Department of Chemistry.
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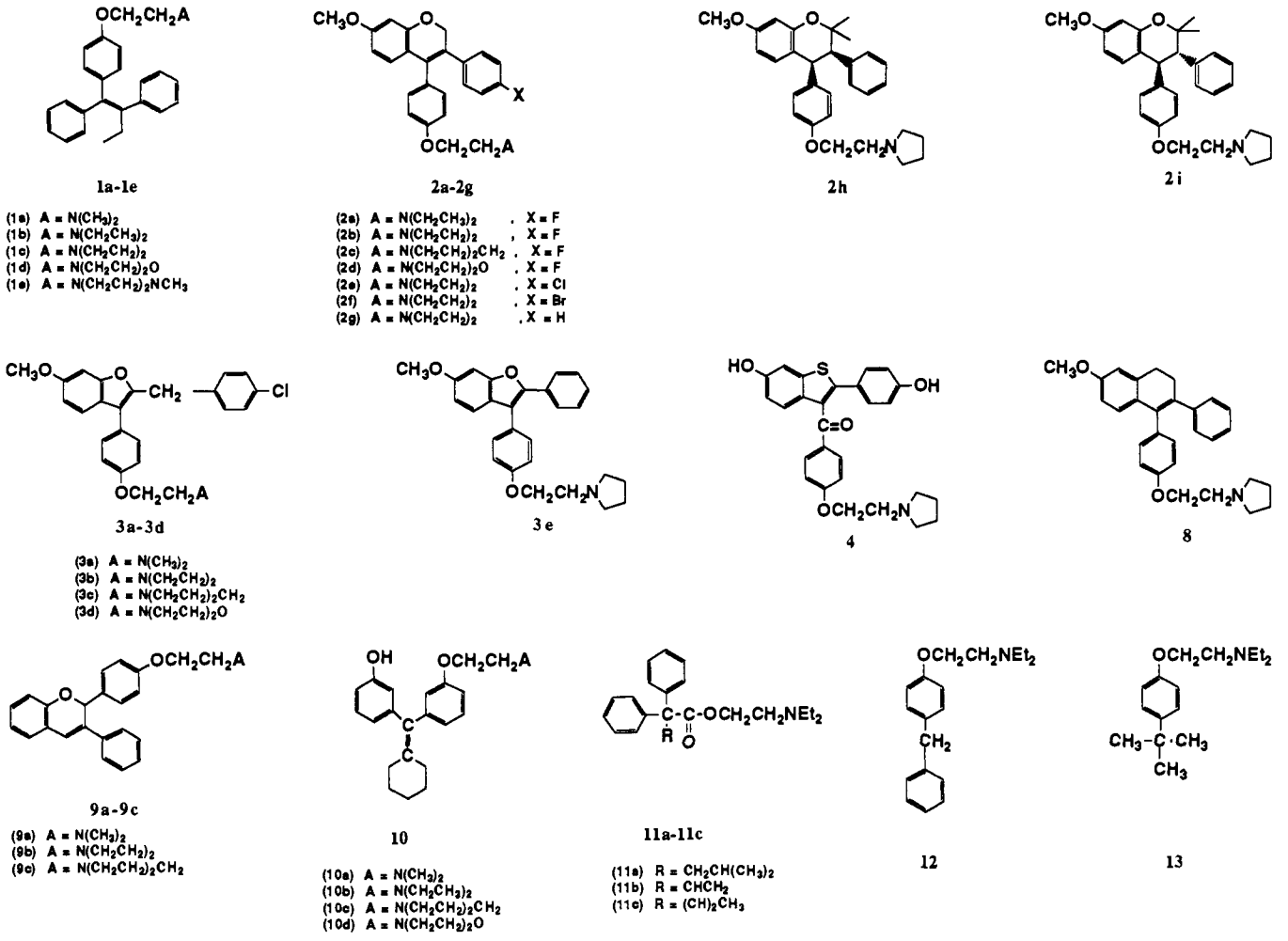
Chart I



rivatives and cytochrome P450 inhibitors) for their ability to bind to AEBS and to inhibit cell proliferation. Three separate studies to date have each shown a positive correlation between binding affinity for AEBS and potency of growth inhibition by chemically disparate compounds.[11,15,17] These correlations, taken in the context of antiestrogen action in AEBS- and ER-deficient cell lines,[2,10] suggest a functional link between ligand binding to AEBS and subsequent growth inhibition. The biochemical basis for such a putative link is presently obscure.

Compounds which bind AEBS with a high degree of selectivity and affinity may be templates for the development of new antitumor agents. While a small number of selective ligands for AEBS has been synthesized,[9,13,16] few bind with high affinity. We therefore undertook to synthesize compounds having a combination of selectivity and high affinity in order to examine their antiproliferative activity in tumor cell lines. We have also studied selected compounds in this series for their effect on cholesterol biosynthesis—a process known to be inhibited by antiestrogens.[18,19] Indeed, antiestrogens have a clinically well-documented hypocholesterolemic effect.[20] Antiestrogens inhibit several steps in the biosynthetic pathway.[18,19,21] Furthermore, inhibition at certain biosynthetic steps occurs in the absence of ER while inhibition at other steps requires ER.[19,21] Although the possibility of AEBS-mediated inhibition of cholesterol biosynthesis is speculative, it may provide a ready explanation for the

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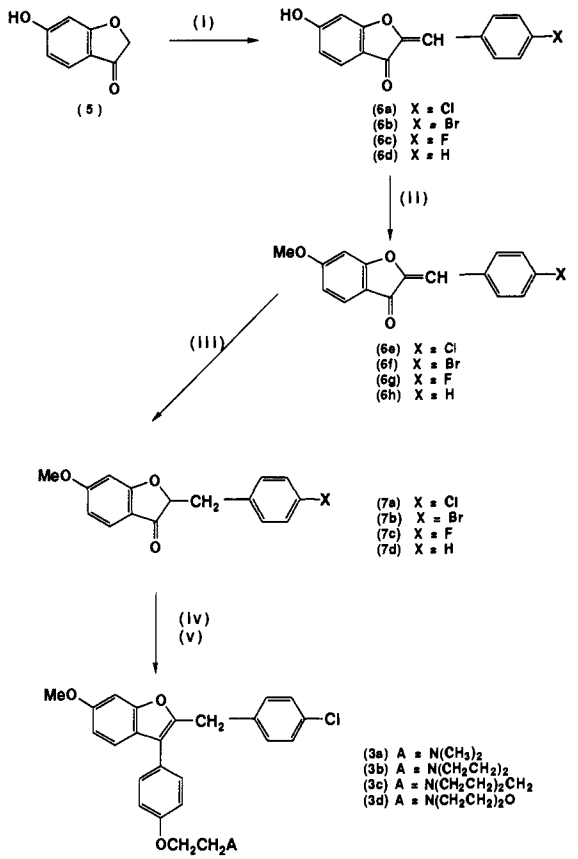
Scheme I[a]



[a] Reagents and conditions: (i) $OHCC_6H_4X$, HCl, EtOH, reflux; (ii) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux; (iii) Pd/H_2, EtOAc; (iv) *p*-$BrC_6H_4OCH_2CH_2A$, Mg, $BrCH_2CH_2Br$, THF, reflux; or *p*-$BrC_6H_4OCH_2CH_2A$, *n*-BuLi, THF, −78 °C; (v) H^+, EtOH, reflux.

mechanism of ER-independent growth inhibition because cholesterol metabolism is closely linked to cell proliferation.[22] This report describes (i) the synthesis of a series of 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans, (ii) the evaluation of their antiproliferative activity in tumor cell lines of different AEBS status, (iii) their effect on de novo cholesterol biosynthesis from acetate, and (iv) the identification of structural features which enhance AEBS binding.

Results and Discussion

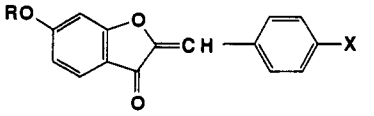
Chemistry. We have recently reported a series of basic ethers of 3-(*p*-fluorophenyl)-4-arylchrom-3-enes[14] (**2a–d**) (Chart I) which were shown to be selective ligands for AEBS in vitro. Following this initial report, we have synthesized a series of brominated, chlorinated and nonhalogenated analogues of the 3,4-diarylchrom-3-enes (**2e–g**), and these analogues have similar biological activity as their fluorinated counterparts.[23]

In an attempt to better define the role of AEBS, we have now extended our studies to a series of compounds having a five-membered benzofuran ring (**3a–d**) (Chart I) in order to ascertain if reduction from a six-membered chromene ring structure (**2a–d**) alters biological activity.

These nonisomerizable 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans (**3a–d**) have several attributes which we envisage would make them ideal lead structures in the development of selective ligands for AEBS. These compounds possess a triarylethylene moiety similar to that also present in nonsteroidal antiestrogens such as **1a**. The presence of an (alkylamino)ethoxy side chain at the 4′-position of the 3-phenyl ring system is critical for antiestrogenic activity.[24] In addition, this class of compounds shares structural similarities with the active benzothiophene antiestrogen, LY117018 (**4**),[25] but are unique in that an additional methylene group is attached at the C-2 position of the benzofuran ring system instead of a carbonyl group at the 3-position of the benzothiophene ring system of LY117018 (**4**).

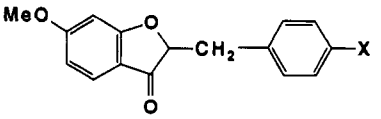
The methodology for the synthesis of the title compounds is depicted in Scheme I. The precursor, 6-hydroxy-3(2*H*)-benzofuranone (**5**) was synthesized via the Hoesch reaction[26] of resorcinol and chloroacetonitrile. Acid-catalyzed condensation[27] of the desired aldehyde with the 6-hydroxy-3(2*H*)-benzofuranone (**5**), in the presence of a catalytic amount of concentrated hydrochloric acid in refluxing ethanol afforded the benzylidenes (**6a–d**) in reasonably good yields (Table I). The benzylidenes were assigned the *Z* configuration (in which the orientation of

Table I. Analytical and Physical Data for **6a–6h**



compd	R	X	mp, °C	yield, %	molecular formula	anal.
6a	H	Cl	271–272	52	$C_{15}H_9O_3Cl$	C,H,Cl
6b	H	Br	240–241	57	$C_{15}H_9O_3Br$	C,H
6c	H	F	253–254	56	$C_{15}H_9O_3F$	C,H,F
6d	H	H	292–293	89	$C_{15}H_{10}O_3$	C,H
6e	Me	Cl	174–175	92	$C_{16}H_{11}O_3Cl$	C,H,Cl
6f	Me	Br	190–192	90	$C_{16}H_{11}O_3Br$	C,H
6g	Me	F	194–195	92	$C_{16}H_{11}O_3F$	C,H
6h	Me	H	149–150	80	$C_{16}H_{12}O_3$	C,H

Table II. Analytical and Physical Data for **7a–7d**



compd	X	mp, °C	yield, %	molecular formula	anal.
7a	Cl	72–73	75	$C_{16}H_{13}O_3Cl$	C,H,Cl
7b	Br	92–93	83	$C_{16}H_{13}O_3Br$	C,H
7c	F	96–97	51	$C_{16}H_{13}O_3F$	C,H,F
7d	H	102–104	97	$C_{16}H_{14}O_3$	C,H

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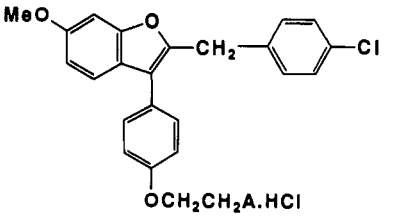
Table III. Analytical and Physical Data for **3a–3d**



compd	A	mp, °C	yield, %	molecular formula	anal.
3a	$N(CH_3)_2$	208–209	23	$C_{26}H_{27}O_3NCl_2$	C,H,N,Cl
3b	$N(CH_2)_4$	223–225	22	$C_{28}H_{29}O_3NCl_2$	C,H,N,Cl
3c	$N(CH_2)_5$	133–136	18	$C_{29}H_{31}O_3NCl_2 \cdot {}^3/_2H_2O$	C,H,N,Cl
3d	$N(CH_2)_4O$	213–214	19	$C_{28}H_{29}O_4NCl_2$	C,H,N

the phenyl ring is trans to the ketone) on the basis of the ^{13}C chemical shift of the exocyclic olefinic carbon atom (=CH) which resonates at δ 109–111 ppm and is consistent with that reported in the literature.[28] ^1H NMR spectra were unsatisfactory for the assignment because the olefinic protons in both *E* and *Z* isomers have been reported to show similar chemical shifts.[28] Before hydrogenation, the benzylidenes were protected with a methyl group, by reaction of the compounds with dimethyl sulfate in acetone using potassium carbonate as the base.

Catalytic hydrogenation of the protected benzylidenes (**6e–h**) with palladium on carbon afforded the 2-benzyl-3(2*H*)-benzofuranones (**7a–d**) in good yields (Table II). Small amounts of side products resulting from competing hydrogenolysis of the halogenated compound and hydrogenation of the carbonyl group were obtained in addition to the desired products. The products were separated by flash chromatography.

Reaction of the 2-benzyl-3(2*H*)-benzofuranones (**7a–d**) with the arylorganomagnesium or arylorganolithium reagent followed by acid-catalyzed dehydration of the tertiary alcohols obtained furnished the title compounds (**3a–d**). The intermediate tertiary carbinol was not isolated. Complete or partial dehydration occurred spontaneously in the course of the Grignard or butyllithium reaction. The benzofurans **3a–d** were purified by flash chromatography on silica gel. The yields and physical data are shown in Table III.

For biological evaluation, the benzofurans were converted to the water-soluble hydrochloride salts which can be prepared at the appropriate stage of the overall synthesis after formation of the tertiary alcohol. These were usually prepared at the final stage either by passing hydrogen chloride gas or by adding a methanolic solution saturated with hydrogen chloride gas to an ethereal solution of the free base.

Biological Activity. The compounds synthesized were evaluated for their ability to (i) compete with [^{3}H]estradiol for binding to ER, (ii) compete with [^{3}H]tamoxifen for binding to AEBS, (iii) suppress the proliferation of cell lines having different levels of AEBS, and (iv) inhibit cholesterol biosynthesis in whole cells.

Ligand Binding. Table IV shows that, compared to diethylstilbestrol, none of the test compounds (present in a 100-fold molar excess) appreciably inhibited [^{3}H]estradiol binding to ER in MCF7 cells, while **1a** slightly displaced bound [^{3}H]estradiol, consistent with its known low affinity for ER.[1] Thus, unlike nonsteroidal antiestrogens, the benzofurans of this series in the present study are not

(28) Pelter, A.; Ward, R. S. Carbon-13 nuclear magnetic resonance spectra of (Z) and (E) aurone. *J. Chem. Soc., Perkin 1* **1979**, 328–329.

Table IV. Competitive Binding to the Estrogen Receptor[a]

compd	bound [^{3}H]estradiol,[b] % of control
1a	82.0 ± 2.0
DES[c]	12.5 ± 3.5
3a	98.0 ± 1.0
3b	99.5 ± 3.5
3c	93.5 ± 0.5
3d	103.5 ± 2.5

[a] Determined by competitive ligand binding assays using MCF7 whole cells as the receptor source. Cells were incubated with 5 nM [^{3}H]estradiol in the absence or presence of a 100-fold excess of DES or the test compounds. The binding reaction was performed and bound [^{3}H]estradiol quantitated as described in the experimental section. [b] Bound [^{3}H]estradiol in the absence of unlabeled competing ligand was set at 100%. Data are the mean ± SEM of two experiments. [c] DES = diethylstilbestrol.

Table V. Competitive Binding to Antiestrogen-Binding Sites[a]

compd	RBA[b]
1a	100
3a	391 ± 195
3b	268 ± 92
3c	605 ± 371
3d	1828 ± 446

[a] Determined by competitive ligand binding assays using EL4 whole cells as the source of AEBS. Cells were incubated with 5 nM [^{3}H]tamoxifen in the absence or presence of an increasing molar excess of unlabeled competing ligand (50–1000 nM). The binding reaction was performed and bound [^{3}H]tamoxifen quantitated as described in the Experimental Section. [b] RBA (relative binding affinity) = (RBA_{50} of **1a**)/(RBA_{50} of test compound) × 100 where RBA_{50} is the concentration required to reduce bound [^{3}H]-tamoxifen by 50%. The data are the mean ± SEM of two experiments.

ligands for ER. However these data do not exclude the possibility that in vitro binding may not reflect in vivo activity of the compounds studied in ER-containing cell types.

In contrast, the summary in Table V shows that all test compounds were more effective than **1a** itself in competing with [^{3}H]tamoxifen for binding to AEBS present in EL4 cells. The binding properties of test compounds were compared to the reference compound **1a**, and were expressed as relative binding affinities (RBA) as defined in Table V. Compound **3d** displayed the highest relative affinity of binding among this series, having an RBA for AEBS 18 times higher than **1a**. Therefore, the benzofuran compounds of this series possess the dual properties of highly selective binding to AEBS (i.e. without interacting with ER) and binding to AEBS with affinities uniformly higher than **1a**—the reference compound for AEBS ligands.

Antiproliferative Activity. The same compounds were next examined for their ability to inhibit cell pro-

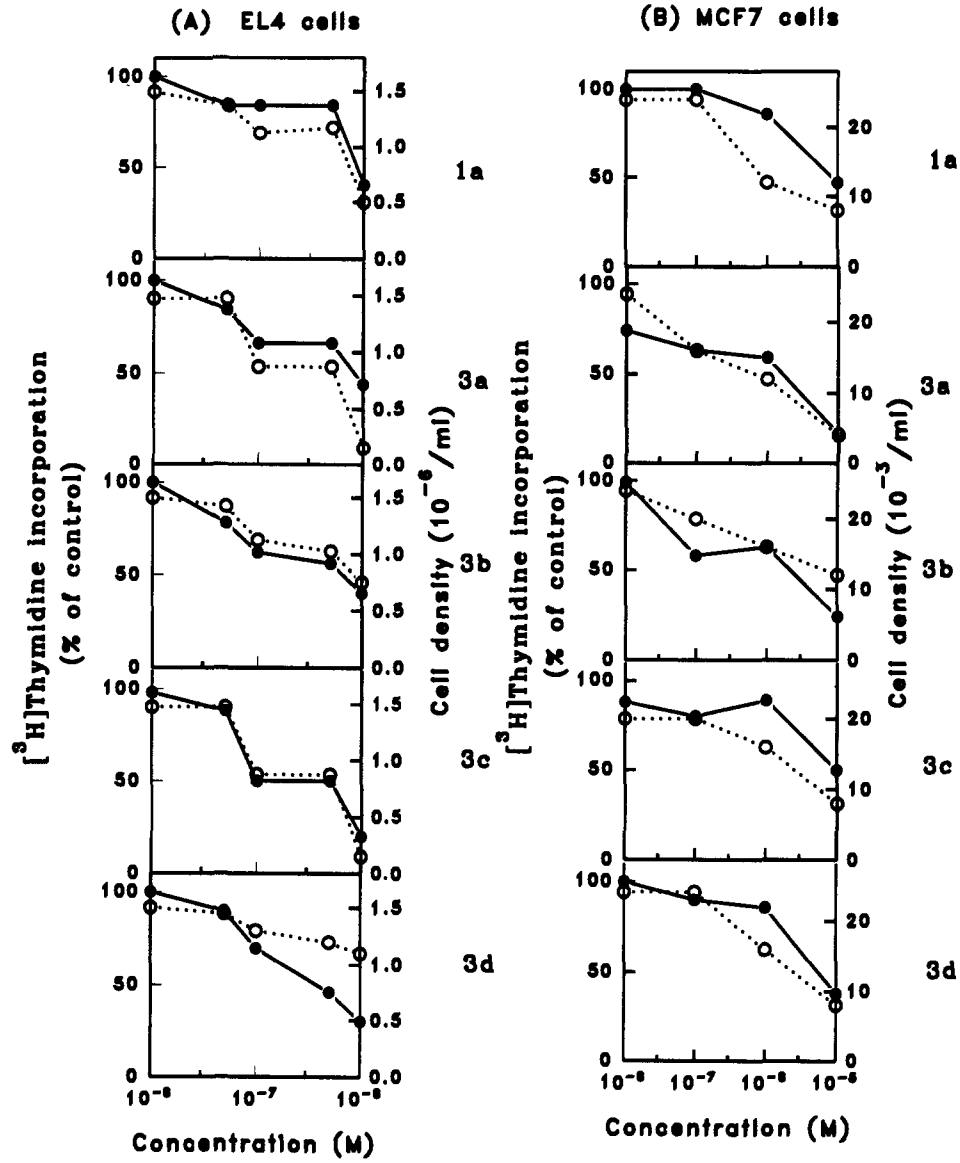


Figure 1. Effect of **1a** and **3a–d** on [^{3}H]thymidine incorporation (●) and cell density (○) by (A) EL4 cells and (B) MCF7 cells. EL4 and MCF7 cells were treated with test compounds for 48 h. [^{3}H]Thymidine incorporation was determined as described in the Experimental Section. Data are the means of two experiments.

liferation as measured by reduction of [^{3}H]thymidine incorporation (i.e. DNA synthesis). Figure 1 shows that, for both EL4 and MCF7 cells, cell densities decreased in parallel with inhibition of [^{3}H]thymidine incorporation. Hence, inhibition of DNA synthesis in these cell lines was a measure of antiproliferative activity. The results of several experiments are summarized in Table VI. It was noted that with the single exception of **3a**, all the other benzofurans were more potent than **1a** in inhibiting proliferation of EL4 cells. This pattern of enhanced antiproliferative activity was also obtained when the same compounds were tested on MCF7 cells, except that all benzofurans were more active than **1a** in this cell line. The IC_{50} (concentration of a compound required to reduce [^{3}H]thymidine incorporation to 50% of its control value) of all compounds (including **1a**) was considerably higher in MCF7 than in EL4 cells (Table VI). It may be relevant that the greater sensitivity of EL4 cells to selective ligands of AEBS is associated with higher AEBS levels in EL4 cells[29] [2277–4538 fmol (mg of protein)$^{-1}$] than in MCF7 cells[7] [355 fmol (mg protein)$^{-1}$].

(29) Ooi, S. O.; Kon, O. L. Unpublished results.

Table VI. Antiproliferative Effect of Benzofurans[a]

compd	IC_{50}, nM[b] EL4	MCF7
1a	535 ± 285	7200 ± 800
3a	950 ± 50	5025 ± 25
3b	475 ± 225	2300 ± 100
3c	300 ± 150	2050 ± 50
3d	335 ± 145	6500 ± 500

[a] Measured as inhibition of [^{3}H]thymidine incorporation by EL4 and MCF7 cells as described in the Experimental Section. [b] IC_{50} is the concentration of a compound required to reduce [^{3}H]thymidine incorporation to 50% of its control value. The data are the mean ± SEM of two experiments.

From Tables V and VI, **3c** and **3d** which had higher relative binding affinities for AEBS than **3a** and **3b** were also more active antiproliferative agents than **1a**. However, when the ranking order for binding affinities and for antiproliferative activities are compared, it is clear that binding affinity is not precisely predictive of biological potency. Specifically **3a** which had higher binding affinity than **1a** was less active than **1a** in the antiproliferative assay. This imperfect correlation can be readily explained in terms of current knowledge of ligand–protein interac-

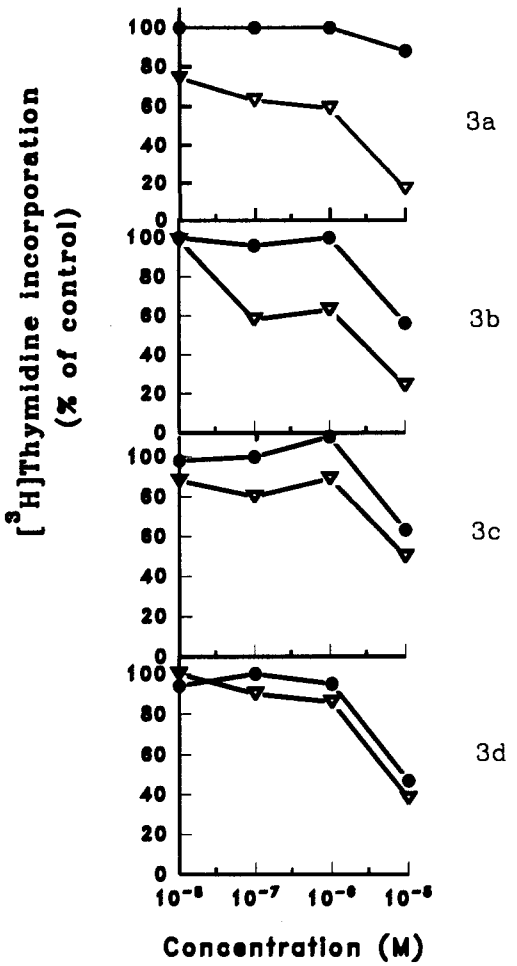


Figure 2. Effect of **3a–d** on [^{3}H]thymidine incorporation by MCF7 (▽) and RTx6 (●) cells. MCF7 and RTx6 cells were treated with 10^{-8}–10^{-5} M of **3a–d** for 48 h. [^{3}H]Thymidine incorporation was determined as described in the Experimental Section. Data are means of two experiments.

tions, such as steroid–receptor binding. Steroid hormone receptor proteins have separate ligand-binding and effector domains.[30] It has been shown that these distinct functions of ligand-binding proteins do not necessarily operate in tandem.[31,32] Such molecular manipulations elegantly confirm theoretical models of drug–receptor interactions which have been proposed to consist of two distinct (though frequently interrelated) components, i.e. binding specificity and effector specificity.[33] By analogy, it can be predicted that different functional classes of ligands may exist for any ligand-binding protein, such as AEBS. Compounds **3c** and **3d** exemplify a class characterized by high binding and effector specificities while **3a** is characteristic of a different functional class having high binding specificity but lower effector specificity.

To determine if AEBS are required for manifestation of the antiproliferative effect of benzofurans, we took advantage of an AEBS-deficient cell line, RTx6. RTx6 was derived as a variant of MCF7 cells from which it differs in lacking AEBS but which it resembles in having equivalent ER levels.[10] The effects of increasing concentrations of **3a–d** on the proliferation of MCF7 and RTx6 cells are summarized in Figure 2. Compound **3a** significantly inhibited DNA synthesis by MCF7 cells at concentrations between 10^{-8}–10^{-5} M ($p < 0.05$). Compound **3b** significantly inhibited DNA synthesis by MCF7 cells at concentrations between 10^{-7}–10^{-5} M ($p < 0.05$). In contrast, 10^{-8}–10^{-6} M of compounds **3a–d** had no effect on RTx6 cells whose incorporation of [^{3}H]thymidine was decreased only at the highest dose tested i.e. 10^{-5} M. These two cell lines also showed, to a lesser extent, differential sensitivity to inhibition by **3c**. The response of MCF7 and RTx6 cells to **3d** was essentially similar. While this latter compound had high RBA for AEBS (Table V) and high antiproliferative activity in EL4 cells (Table VI), its lower activity in MCF7 cells is consistent with subtle differences in AEBS properties in different cell types. Given that RTx6 cells are deficient in AEBS and that the benzofurans are selective ligands for AEBS, these data implicate AEBS in expression of the antigrowth effect.

Table VII. Effect of **3b** on Cholesterol Biosynthesis by EL4 Cells

compd	% total radioactivity incorporated as cholesterol[c] I[f]	II[f]	mean	% inhibition of cholesterol synthesis	% cell viability[e]
control[a]	0.20	0.22			
	0.25	0.23	0.23 ± 0.02		96
	0.26	0.24			
3b[a]	0.13	0.11			
	0.10	0.12	0.12 ± 0.01	48	91
	0.12	0.14		($p < 0.01$)[d]	
control[b]	0.34	0.28			
	0.32	0.30	0.32 ± 0.02		92
	0.36	0.32			
3b[b]	0.16	0.14			
	0.15	0.17	0.17 ± 0.02	47	87
	0.24	0.14		($p < 0.01$)[d]	

[a] EL4 cells (20×10^6 cells/mL in well) were incubated with 0.25 mM [^{3}H]acetate in the absence or presence of **3b** (10^{-6} M) for 5 h. [b] EL4 cells (20×10^6 cells/mL in well) were incubated with 0.25 mM [^{3}H]acetate in the absence or presence of **3b** (10^{-7} M) for 48 h. [c] (Radioactivity incorporated as cholesterol)/(Total radioactivity incorporated by cells) × 100. [d] p value was obtained by applying Student's t test. [e] Cell viability was estimated by trypan blue exclusion. [f] Data from two experiments (each performed in triplicate).

Table VIII. Effect of **3d** on Cholesterol Biosynthesis by EL4 Cells

compd	% total radioactivity incorporated as cholesterol[b] I[e]	II[e]	mean	% inhibition of cholesterol synthesis	% cell viability[d]
control[a]	0.25	0.24			
	0.24	0.27	0.25 ± 0.02		96
	0.20	0.28			
3d[a]	0.19	0.11			
	0.09	0.12	0.11 ± 0.03	56	79
	0.05	0.11		($p < 0.05$)[c]	

[a] EL4 cells (20×10^6 cells/mL in well) were incubated with 0.25 mM [^{3}H]acetate in the absence or presence of **3d** (10^{-4} M) for 5 h. [b] (Radioactivity incorporated as cholesterol)/(total radioactivity incorporated by cells) × 100. [c] p value was obtained by applying Student's t test. [d] Cell viability was estimated by trypan blue exclusion. [e] Data from two experiments (each performed in triplicate).

Cholesterol Biosynthesis. Tables VII and VIII show that EL4 cells incorporated [^{3}H]acetate into cholesterol

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and that **3b** and **3d** significantly inhibited this process. Inhibition of cholesterol biosynthesis by both compounds was relatively rapid in onset—requiring only 5 h of treatment by 10^{-6} M of **3b** (Table VII) and 10^{-4} M of **3d** (Table VIII). As both compounds were antiproliferative and potentially cytotoxic, it was necessary to exclude the possibility that the observed effects on cholesterol biosynthesis did not merely result from loss of viable cells in test incubations. That this was not the case is indicated by a similar percentage of viable cells in the absence or presence of each benzofuran compound (Table VII). Although the use of a higher concentration (10^{-4} M) of **3d** was associated with some decrease of cell viability relative to control cells (96% control vs 79% treated cells) (Table VIII), there was nonetheless a disproportionately greater decrease of [^{3}H]acetate incorporation into [^{3}H]cholesterol (56% inhibition) which could not be explained on the basis of loss of viable cells alone.

These results demonstrate the ability of selective AEBS ligands to rapidly decrease cholesterol biosynthesis in a manner which precedes their ability to inhibit DNA synthesis and to cause cell death. Inhibition of cholesterol biosynthesis by these compounds is estrogen receptor-independent since EL4 cells lack estradiol-binding sites. However, the present studies have not addressed the question of involvement of AEBS in mediating this effect.

Whatever the mechanism of action, interference with cholesterol synthesis provides a possible explanation for the antiproliferative and cytotoxic activity of the chromenes,[14] benzofurans, and nonsteroidal antiestrogens on lymphoid cells.[11,12] Normal cholesterol synthesis is a prerequisite for proliferation and growth of cells not only because it is required for membrane structures but also because biosynthetic precursors of cholesterol, such as isoprenoids, dolichol and other unidentified nonsterol intermediates, have key roles in a variety of cellular functions.[22]

Structural Features Which Enhance Binding to the Antiestrogen-Binding Sites. The structural requirements for binding to the antiestrogen-binding sites by the 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans (**3a–d**) are compared to a series of the following compounds reported in the literature: (a) analogues of triarylethylene antiestrogens;[6,16] (b) analogues of chromenes,[14,23] *cis*-chroman,[35] and centchroman;[35] (c) 2-phenyl-3-[*p*-(2-pyrrolidinoethoxy)phenyl]-6-methoxybenzofuran;[35] (d) benzothiophene (LY117018)[6] and dihydronaphthalene (nafoxidine);[34] (e) 2,3-diaryl-2*H*-1-benzopyrans;[36,37] (f) cyclofenil;[16] (g) SKF analogues;[16] (h) *N,N*-diethyl-2-[(4-phenylmethyl)phenoxy]ethanamine hydrochloride (DPPE),[13] and (i) [(*tert*-butylphenoxy)ethyl]diethylamine (BPEA).[9]

The relative binding affinities (RBA) to AEBS and ER of the above classes of compounds are tabulated in Table IX. Tamoxifen and estradiol are used as reference compounds for comparison for binding to AEBS and ER, respectively. That different tissue sources of AEBS were used by different laboratories introduces a possible caveat to these comparisons because AEBS from different tissue sources may have subtle differences in their ligand-binding sites.[34]

Table IX. Relative Binding Affinities (RBA) of AEBS Ligands

compound	AEBS[g]	ER[h]
	(a) Triphenylethylenes	
1a[6]	100[d]	2[d]
1b[16]	21,[d] 46[a]	
1c[6]	434[d]	7.6[d]
1d[6]	1174[d]	0.2[d]
1e[6]	540[d]	10.5[d]
	(b) Chromenes and Chromans	
2a[14]	106[c]	<1[a]
2b[14]	176[c]	<1[a]
2c[14]	161[c]	<1[a]
2d[14]	68[c]	<1[a]
2e[23]	257[b]	<1[a]
2f[23]	169[b]	<1[a]
2g[23]	557[b]	<1[a]
2h[35]	46[d]	0.001[d]
2i[35]	19[d]	5.2[d]
	(c) Benzofurans	
3a	586[b]	<1[a]
3b	360[b]	<1[a]
3c	976[b]	<1[a]
3d	2274[b]	<1[a]
3e[35]	23[d]	0.01[d]
	(d) Benzothiophene and Dihydronaphthalene	
4[6]	19[a]	115[a]
8[34]	183,[e] 90,[d], 124[f]	
	(e) 2,3-Diaryl-2*H*-1-benzopyrans	
9a[36]	98[d]	0.11[d]
9b[36]	134[d]	0.5[d]
9c[36]	225[d]	0.3[d]
	(f) Cyclofenil	
10a[16]	17[d]	
10b[16]	6[d]	
10c[16]	10[d]	
10d[16]	25[d]	
	(g) SKF Analogues	
11a[16]	5[d]	
11b[16]	10[d]	
11c[16]	12[d]	
	(h) DPPE (i) BPEA	
12[13]	0.06[d]	<0.003[d]
13[9]	0.08[d]	<0.003[d]

[a] MCF-7. [b] EL-4. [c] N1-S1 cells. [d] Rat liver. [e] Chicken liver. [f] Guinea pig uterus. [g] RBA of tamoxifen = 100. [h] RBA of estradiol = 100.

Compounds **3a–d** have a much higher binding affinity for AEBS than the triarylethylenes **1a–e**. The triarylethylene derivative with a morpholino ring structure[16] (**1d**) has a very high binding affinity compared to analogues with other basic side chains. This is consistent with what was observed for **3a–d**, in which **3d** having a morpholino ring structure has the highest binding affinity. The chromenes[14] **2a–g** have relatively similar binding affinities to **3a–d** showing that reducing the ring size of the six-membered chromene ring system (**2**) to the five-membered benzofuran ring system (**3**) does not change binding affinity. *cis*-Chroman (**2h**)[35] and centchroman (**2i**)[35] have lower binding affinities than the chromenes **2a–g** and benzofurans **3a–d**, suggesting that the presence of an unsaturated carbon–carbon double bond enhances binding to AEBS. In comparison to the benzofuran without a

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methylene group at the C-2 position (**3e**),[35] **3a–d** have a much higher affinity for AEBS suggesting that a methylene group at the C-2 position of the benzofuran ring system enhances affinity.

2,3-Diaryl-2*H*-1-benzopyrans (**9a–c**) bind comparably well to AEBS[36,37] as **3a–d**. The presence of a nonisomerizable triarylethylene system in a fixed heterocyclic oxygen ring common to both classes of compounds may contribute to high affinity binding.

Other classes of compounds such as the cyclofenils[16] (**10a–e**), SKF analogues[16] (**11a–c**), DPPE[13] (**12**), and BPEA[9] (**13**) which lack the triarylethylene moiety have detectable but low binding affinities[16] compared to **3a–d**.

In summary, the high binding affinity of **3a–d** for AEBS may be attributable to the following features: (i) the presence of a triarylethylene moiety in a fixed heterocyclic oxygen ring system, (ii) the presence of an (alkylamino)-ethoxy side chain at the 4′-position of the 3-phenyl ring, and (iii) the presence of an additional methylene group at the C-2 position of the benzofuran ring.

Experimental Section

1. **Chemistry.** All melting points are uncorrected and were determined with a Thomas Hoover apparatus. IR spectra were recorded with a Perkin-Elmer 1310 infrared spectrophotometer. ^{1}H NMR spectra were obtained at 90 MHz with a JEOL FX90Q FT NMR spectrometer (internal reference $SiMe_4$, and $CDCl_3$ as solvent unless indicated). NMR data are presented as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; bs, broad singlet), number of protons, coupling constant(s) in Hertz. Carbon ^{1}H-decoupled ^{13}C NMR spectra were obtained at 75.46 MHz with a Bruker ACF300 NMR spectrometer [internal reference $CDCl_3$ (77.0 ppm), $CDCl_3$ as solvent unless indicated]. Mass spectra were performed on a VG 7035 micromass mass spectrometer at a source temperature of 200 °C and an ion current of 70 eV. Elemental analyses were performed on a Perkin-Elmer Model 240C elemental analyzer and were within ±0.4% of calculated values. Analytical thin-layer chromatography (TLC) was performed on Polygram precoated plastic sheets of silica gel 60 and inspected under ultraviolet radiation (UV_{254}). Flash chromatography was performed according to the method of Still[39] using 0.040–0.063-mm silica gel.

6-Hydroxy-3(2*H*)-benzofuranone (5). This compound was synthesized from resorcinol and chloroacetonitrile following a similar procedure[40] used for the preparation of 4,6-dihydroxy-3-(2*H*)-benzofuranone: yield (48%); mp 240 °C dec (lit.[41] mp 240 °C dec).

2-(*p*-Chlorobenzylidene)-6-hydroxy-3(2*H*)-benzofuranone (6a). A mixture of 6-hydroxy-3(2*H*)-benzofuranone (12.8 g, 86 mmol), *p*-chlorobenzaldehyde (12.7 g, 90.0 mmol), and concentrated hydrochloric acid (1 mL) in absolute ethanol (150 mL) was heated at 60–70 °C for 3 h. Orange precipitate formed, was filtered, and recrystallized from ethanol to yield orange glistening plates of **6a** (13.6 g, 52%): mp 271–272 °C dec; IR ν(max) (Nujol) 3500 (OH), 1670 (C=O), 1630 (C=C) cm^{-1}; ^{1}H NMR δ (ppm) ($CDCl_3$ + DMSO-d_6) 3.86 (bs, OH, disappeared with D_2O exchange), 6.65–6.75 (m, 3 H, H-5, H-7, and C=C—H), 7.40–7.59 (m, 5 H, ArH); ^{13}C NMR δ (ppm) 182.65, 168.53, 166.65, 148.14, 135.07, 132.28, 13 2.05, 131.07, 128.99, 128.41, 126.00, 119.07, 113.07, 109.53, 98.49; MS *m/z* 274, 272 (M$^+$, 25, 67), 271 (100), 237 (28), 136 (20). Anal. ($C_{15}H_9O_3Cl$) C, H, Cl.

A similar procedure to that described for **6a** was used for the preparation of **6b–d** and the yields and physical constants are given in Table I.

2-(*p*-Chlorobenzylidene)-6-methoxy-3(2*H*)-benzofuranone (6e). A mixture of compound **6a** (4.0 g, 14.7 mmol), dimethyl sulfate (1.5 mL, 15.4 mmol), and anhydrous potassium carbonate (7.1 g 51.5 mmol) in dry acetone (100 mL) was refluxed for 3 h, after which anhydrous potassium carbonate was filtered off and the filtrate was evaporated to give a residue. Addition of distilled water (100 mL) to the oil and trituration of the cooled mixture gave yellow crystals which were recrystallized from methanol to yield **6e** (4.2 g, 92%): mp 174–175 °C; IR ν(max) (Nujol) 1700 (C=O), 1660 (C=C) cm^{-1}; ^{1}H NMR δ (ppm) 3.93 (s, 3 H, OCH_3), 6.75 (m, 3 H, methine H at C-2, H-5, and H-7), 7.40–7.65 (m, 5 H, ArH); ^{13}C NMR δ (ppm) 182.78, 168.51, 167.56, 147.92, 135.50, 132.38, 130.96, 129.11, 125.89, 114.71, 112.30, 110.35, 96.69, 56.06; MS *m/z* 288, 286 (M$^+$, 25, 56), 285 (100), 251 (20), 150 (15). Anal. ($C_{16}H_{11}O_3Cl$) C, H, Cl.

A similar procedure to that described for **6e** was used for the preparation of **6f–h** and the yields and physical constants are given in Table I.

2-(*p*-Chlorobenzyl)-6-methoxy-3(2*H*)-benzofuranone (7a). Compound **6e** (2.0 g, 6.98 mmol) in ethyl acetate (150 mL) was hydrogenated at room temperature and atmospheric pressure over 10% Pd on carbon (400 mg) until the hydrogen uptake was (175 mL, 6.98 mmol). Palladium was filtered off through Celite and the solvent was evaporated off to give a colorless mixture of three products which was separated by flash chromatography on silica (eluent, chloroform/hexane = 1:1). The desired product was recrystallized from methanol yielding white crystals of **7a** (1.5 g, 75%): mp 72–73 °C; ^{1}H NMR δ (ppm) 2.83–3.43 (2q, 2 H, CH_2 at C-2), 3.85 (s, 3 H, OCH_3), 4.75 (dd, 1 H, H-2), 6.47–7.56 (m, 7 H, ArH); MS *m/z* 290, 288 (M$^+$, 16, 48), 254 (15), 177 (35), 125 (109), 91 (27). Anal. ($C_{16}H_{13}O_3Cl$) C, H, Cl.

A similar procedure to that described for **7a** was used for the preparation of **7b–d** and the yields and physical constants are given in Table II.

2-(*p*-Chlorobenzyl)-3-[p-[2-(dimethylamino)ethoxy]-phenyl]-6-methoxybenzofuran Hydrochloride (3a). *n*-Butyllithium (5.27 mL, 7.80 mM) in THF was added dropwise to a solution of 4-[2-(dimethylamino)ethoxy]phenyl bromide (1.90 g, 7.80 mmol) in THF (5 mL) at −78 °C. After the mixture was stirred for 30 min at −78 °C, **7a** (1.50 g, 5.20 mmol) in THF (12 mL) was added dropwise to the mixture at −78 °C. The reaction mixture was stirred at −78 °C for 1 h and allowed to warm to room temperature. After stirring for 18 h, it was quenched with saturated ammonium chloride and THF was evaporated off. The residue was dissolved in ethyl acetate (30 mL), washed with brine (2 × 20 mL), and dried (anhydrous magnesium sulfate), and the solvent was evaporated off to yield an oil which was purified by flash chromatography (eluent, *n*-hexane/dichloromethane/triethylamine = 1:1:0.2), yielding a light brown oil of the tertiary alcohol which without characterization was dehydrated. A solution of methanol (0.4 mL) saturated with dry hydrogen chloride gas was added to the tertiary alcohol, dissolved in ether (40 mL). A white precipitate was formed which was filtered and recrystallized from ethanol to give **3a** as a white crystalline solid (23%): mp 208–290 °C; ^{1}H NMR δ (ppm) 2.96 (s, 6 H, $N(CH_3)_2$), 3.48 (t, 2 H, J = 6.0 Hz, OCH_2CH_2N), 3.85 (s, 3 H, OCH_3), 4.10 (s, 2 H, CH_2 at C-2), 4.59 (t, 2 H, J = 6.0 Hz, OCH_2CH_2N), 6.81–7.45 (m, 11 H, Ar*H*); MS *m/z* 437, 435 (M$^+$ − HCl, 7, 23), 364 (12), 58 (100). Anal. ($C_{26}H_{26}O_3NCl$·HCl) C, H, N, Cl.

2-(*p*-Chlorobenzyl)-3-[*p*-(2-pyrrolidinoethoxy)phenyl]-6-methoxybenzofuran Hydrochloride (3b). 4-[2-Pyrrolidinoethoxy]phenyl bromide (1.7 g, 6.2 mmol) in dry THF (50 mL) was added to magnesium turnings (240 mg, 10 mmol) in a dry three-necked round-bottom flask under an oxygen-free nitrogen atmosphere, and the mixture was maintained under gentle reflux. 1,2-Dibromoethane (0.27 mL, 3.12 mmol) was added dropwise over a period of 0.5 h. The mixture was heated gently until the magnesium was nearly consumed (ca. 0.5 h) and **7a** (740 mg, 2.6 mmol) in THF (50 mL) was added dropwise to the mixture at 0 °C. The reaction mixture was allowed to warm to room temperature and refluxed for 18 h. The cooled mixture was quenched with saturated ammonium chloride (30 mL), extracted with dichloromethane (3 × 40 mL), washed with water, and dried

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(anhydrous sodium sulfate), and the solvent was evaporated off to yield an oil which was dehydrated with concentrated hydrochloric acid (2 mL) in ethanol (25 mL). The mixture was refluxed for 2 h, whereupon ethanol was removed. It was then basified with 10% sodium hydroxide (20 mL) and extracted with dichloromethane (3 × 40 mL). The dichloromethane extract was washed with water (3 × 20 mL) and dried (anhydrous sodium sulfate), and the solvent was evaporated off to give a solid which was chromatographed on silica. Elution with (dichloromethane/hexane/triethylamine = 1:2:0.3) yielded a colorless solid which was recrystallized from methanol to give the free base of **3b**, mp 75 °C. Dry hydrogen chloride gas was bubbled through an ethereal solution of the free base for 10 mins and cooled to 0 °C. The hydrochloride salt which precipitated out was filtered off at the pump, rinsed with ether, and recrystallized from 2-propanol to yield **3b**: yield 22%; mp 223–225 °C; ^{1}H NMR δ (ppm) 1.82 (m, 4 H, N(CH$_2$C*H*$_2$)$_2$ (ring)), 2.65 (br s, 4 H, N(C*H*$_2$CH$_2$)$_2$ (ring)), 2.93 (t, 2 H, J = 6.0 Hz, OCH$_2$C*H*$_2$N), 3.84 (s, 3 H, C*H*$_3$O), 4.16 (distorted t, 4 H, J = 6.0 Hz, OC*H*$_2$CH$_2$N, C*H*$_2$ at C-2), 6.80–7.47 (m, 11 H, Ar*H*), MS m/z 463, 461 (M^+ – HCl, 28, 10), 364 (17), 98 (38), 84 (100). Anal. ($C_{28}H_{28}O_3NCl$·HCl) C, H, N, Cl.

A similar procedure to that described for **3a** was used for the preparation of **3c–d** and the yields and physical constants are given in Table III.

2. Biological Methods. Materials. [*N-methyl*-^{3}H]Tamoxifen (321.9 GBq/mmol) and [*methyl*-^{3}H]thymidine (247.9 GBq/mmol) were from New England Nuclear. [2,4,6,7-^{3}H]Estradiol (93.34 TBq/mmol), [^{3}H]acetic acid, sodium salt (103 GBq/mmol), and [4-^{14}C]cholesterol (2.04 GBq/mmol) were from Amersham International. Tamoxifen citrate, clomiphene citrate, diethylstilbestrol, Hepes-buffered RPMI 1640 medium, and antibiotics were from Sigma Chemical Company. Mycoplasma-free newborn calf serum was from Gibco-BRL. Cholesterol and all other chemicals were from E. Merck and were of analytical grade or better.

Cell Lines and Maintenance. EL4, a lymphoid cell line (gift from Dr. K. M. Hui, National University of Singapore), was propagated in lipoprotein-poor, Hepes-buffered RPMI 1640 medium (LP-RPMI) prepared as previously described.[11]

Cell viability was estimated by trypan blue exclusion. All experiments were initiated with cultures containing 90% or more viable cells.

MCF7, a human breast cancer-derived cell line (American Type Culture Collection, MD) was grown in Hepes-buffered RPMI 1640 medium supplemented with 5% newborn calf serum, penicillin (100 000 U/L) and streptomycin (100 mg/L). Medium was renewed at two-day intervals. Cultures were trypsinized at confluence (0.05% trypsin/0.53 mM EDTA) and subcultured at 100 000 cells/mL.

An AEBS-deficient cell line, RTx6 (a gift from Professor J. C. Faye of C.H.U. Rangueil, Toulouse, France), was propagated and maintained in identical manner as MCF7 except that 10^{-6} M tamoxifen was present in the growth medium until 24 h before use in the antiproliferative assays.

Ligand Binding (AEBS). Relative binding affinities (RBA) were determined by competitive ligand binding assays using [^{3}H]tamoxifen and EL4 whole cells suspended in phosphate-buffered saline (0.14 M NaCl, 0.0034 M KCl, 0.012 M Na_2HPO, 0.00174 M KH_2PO_4, pH 7.4). Duplicate aliquots of EL4 cells (3 × 10^7 cells in 0.2 mL) were incubated with 5 nM [^{3}H]tamoxifen and an increasing molar excess of unlabeled competing ligand (50–1000 nM). Nonspecific binding was obtained in the presence of a 200-fold excess of unlabeled tamoxifen and averaged 22% of total binding. Binding occurred overnight at 4 °C and was terminated by addition of 1.5 mL phosphate-buffered saline and centrifugation (2000g for 10 min at 4 °C). Supernatants were decanted to waste and each cell pellet was washed free of unbound [^{3}H]tamoxifen with 0.5% Tween 80 in phosphate-buffered saline (two washes of 2 mL each). Washed cell pellets were transferred to Beckman Ready Caps, dried according to the manufacturer's instructions and counted.

Ligand Binding (ER). The ability of test compounds to bind to ER was determined using MCF7 whole cells suspended in phosphate-buffered saline. Duplicate aliquots (3 × 10^7 cells in 0.4 mL) were incubated with 5 nM [^{3}H]estradiol in the absence or presence of a 100-fold molar excess of diethylstilbestrol or the test compounds. The binding reaction was terminated and bound [^{3}H]estradiol quantitated as described above.

Antiproliferative Assays. This was performed on EL4 cells by quantitating [^{3}H]thymidine incorporation using a previously described method[11] except that cells were harvested with a Brandel Harvester (M-24), trapped on Beckman Ready Filter strips and counted in a Beckman LS 3801 counter. [^{3}H]Thymidine incorporation by MCF7 and RTx6 cells was determined by dispensing cells into a 96-well plate (4500 cells, 0.18 mL per well). Test compounds were diluted from 1 mM ethanolic stock solutions to yield final concentrations between 10^{-6} and 10^{-6} M. Control wells received solvent alone. After 24 h, test compounds (0.02 mL per well) and [^{3}H]thymidine (37 kBq, 0.02 mL per well) were added. Following another 48-h incubation, cells were trypsinized (0.05 mL of 0.05% trypsin/0.53 mM EDTA per well), harvested and counted as described above.

IC_{50} is the concentration of a compound required to reduce [^{3}H]thymidine incorporation to 50% of its control value.

Cell counts were performed manually using a Neubauer hemocytometer.

Cholesterol Biosynthesis. Quantitation of cholesterol synthesis from labeled acetate was based on the method of Ho et al.[42] EL4 cells in LP-RPMI were spun for 5 min and resuspended in fresh LP-RPMI at a final concentration of 20 × 10^6 cells/mL. Triplicate aliquots of the cell suspension (0.8 mL) were incubated in wells containing [^{3}H]acetic acid, sodium salt (0.10 mL, 0.25 mM), and the test compound (0.10 mL, 10^{-4}–10^{-6} M).

The incubations were terminated by addition of potassium hydroxide (0.25 mL, 50%) and ethanol (0.5 mL). [^{14}C]Cholesterol (0.01 mL, 1 μCi) was added to each well as an internal standard to calculate recovery. After saponification at 95 °C for 90 min, 1 mL of ethanol was added. Nonsaponifiable lipids were extracted with petroleum ether (40–60 °C) (3 × 10 mL) and the pooled ether extracts were back-washed with sodium hydroxide (0.1 M, 2 mL), followed by distilled water (2 mL). The petroleum ether fraction was transferred to a round-bottomed flask and concentrated under vacuum with a Buchi rotary evaporator. The concentrate was transferred with ethanol (2 × 1 mL) to a small test tube and evaporated to dryness under nitrogen. The dried residue was redissolved in a known volume of ethanol and an aliquot (20 μL) was applied to Mylar-backed silica TLC sheets. Authentic unlabeled cholesterol was spotted in an adjacent lane and the chromatogram was developed in chloroform.

Lipids were visualized on dried chromatograms with iodine vapor and those having the same R_f value as authentic cholesterol were scraped and extracted with ethanol (2 × 1 mL). An aliquot (200 μL) of the ethanol extract was transferred to Beckman Ready Caps, dried, and counted as described above. Data are expressed as percentage inhibition of [^{3}H]acetate incorporation into [^{3}H]-cholesterol, with reference to control cell incubations in which the proportion of total radioactivity [^{3}H] converted to labeled cholesterol is regarded as 100%.

Statistical Analysis. The significance of differences between means was assessed by Student's t-test.

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Registry No. **3a**·HCl, 139276-06-5; **3a** free base, 139276-10-1; **3b**·HCl, 139276-07-6; **3b** free base, 139276-11-2; **3c**·HCl, 139276-08-7; **3c** free base, 139276-12-3; **3d**·HCl, 139276-09-8; **3d** free base, 139276-13-4; **5**, 6272-26-0; **6a**, 139276-14-5; **6b**, 139276-15-6; **6c**, 139311-84-5; **6d**, 139276-16-7; **6e**, 139276-17-8; **6f**, 139276-18-9; **6g**, 139276-19-0; **6h**, 31356-11-3; **7a**, 139276-20-3; **7b**, 139276-21-4;

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7c, 139276-22-5; 7d, 108837-12-3; resorcinol, 108-46-3; chloroacetonitrile, 107-14-2; *p*-chlorobenzaldehyde, 104-88-1; *p*-bromobenzaldehyde, 1122-91-4; *p*-fluorobenzaldehyde, 459-57-4; benzaldehyde, 100-52-7; 4-[2-(dimethylamino)ethoxy]phenyl bromide, 2474-07-9; 4-[2-pyrrolidinoethoxy]phenyl bromide, 1081-73-8; 4-[2-piperidinoethoxy]phenyl bromide, 836-58-8; 4-[2-morpholinoethoxy]phenyl bromide, 836-59-9.

Supplementary Material Available: Analytical, ^1H NMR, and mass spectral data for 2-benzylidene-6-hydroxy-3(2*H*)-benzofuranones (**6b–d**), 2-benzylidene-6-methoxy-3(2*H*)-benzofuranones (**6f–h**), 2-benzyl-6-methoxy-3(2*H*)-benzofuranones (**7b–d**), and 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans (**3c–d**), and ^{13}C NMR spectral data for **6b–d,f–h** (1 page). Ordering information is given on any current masthead page.

2-Substituted 3-(Aminooxy)propanamines as Inhibitors of Ornithine Decarboxylase: Synthesis and Biological Activity

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1-Amino-3-(aminooxy)-2-propanol (**6a**) has been synthesized and found to inhibit rat liver ornithine decarboxylase (ODC) with an IC_{50} in the nanomolar range. Compound **6a** served as a basis for the design of new enzyme inhibitors, which led to the identification of 3-(aminooxy)-2-fluoropropanamine (**15**) as a new powerful enzyme blocker. Compound **15** inhibited ODC at 3 times lower concentrations than **6a** and 3-(aminooxy)propanamine (APA), and it was superior to APA as an antiproliferative agent in inhibiting the growth of human T_{24} bladder carcinoma cells in vitro.

Introduction

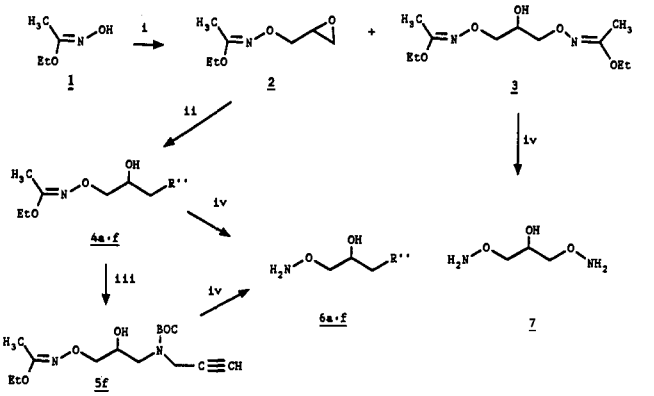
Ornithine decarboxylase (ODC) is one of the rate-limiting enzymes of polyamine biosynthesis.[1] It is present in every mammalian cell and is responsible for the conversion of L-ornithine to putrescine, a precursor of the higher polyamines spermidine and spermine.[2] ODC activity is elevated in rapidly proliferating and neoplastic tissues and is therefore a possible target for inhibition of polyamine metabolism.[3] In an attempt to control polyamine biosynthesis, several substrate and putrescine-based ODC inhibitors were synthesized.[4-6] Furthermore, 3-(aminooxy)propanamine (APA), previously reported as a chemical intermediate for the synthesis of bacteriostatic products,[7] was identified in 1985 as the most potent substrate-competitive inhibitor of ODC in vitro.[8] APA is more potent than the homologous 4-(aminooxy)butylamine[8] or 2-aminooxyethylamine[9] and appears not to inhibit ODC by interaction with the coenzyme pyridoxal phosphate (PP).[10]

The potent biological activity and the simple structure of APA prompted us to synthesize a series of new ODC inhibitors based on 2-substituted 3-(aminooxy)-1-propanamines and evaluate their biological activity. The aim of our study was to elucidate the structural requirements for selective ODC inhibition and to find new, more potent drugs. In the present article, we describe the work leading to the identification and first characterization of 3-(aminooxy)-2-fluoropropanamine (**15**) as a new lead compound.

Chemistry

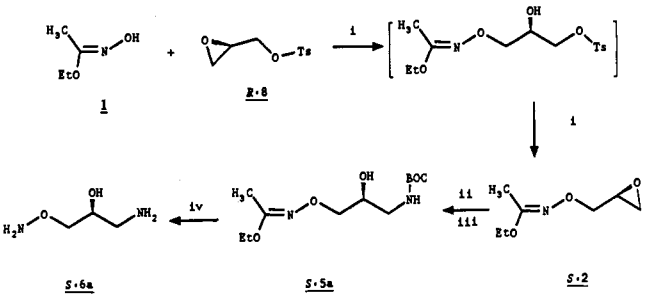
The racemic 3-(aminooxy)-2-propanols **6a–f** and the achiral 1,3-bis(aminooxy)-2-propanol **7** were synthesized, with some modifications, according to the published procedure for **6a**, **6d**, and **7**[11] (Scheme I). Instead of treating the dry sodium salt of **1** with a large excess of boiling epichlorohydrin, **1** was alkylated in acetone solution with 2 equiv of epichlorohydrin, during slow addition of 10 N NaOH, to give an easily separable mixture of **2** and **3**. Our procedure is easy to perform and affords pure **2** in a satisfactory 46% yield. Compound **2** reacted with concentrated ammonium hydroxide and different amines, leading to the 2-propanolamines **4a–f**. The intermediate **4a** was isolated by crystallization of its hydrochloride salt, whereas

Scheme I[a]



[a] (i) Epichlorohydrin, 10 N NaOH, acetone, 60 °C, 20 h; (ii) concentrated NH_4OH or amines, 20–85 °C, 5–18 h; (iii) $(BOC)_2O$, CH_2Cl_2; (iv) 2 N HCl, reflux, 1 h.

Scheme II[a]



[a] (i) 10 N NaOH, acetone, 60 °C, 20 h; (ii) concentrated NH_4OH, room temperature, 18 h; (iii) $(BOC)_2O$, Na_2CO_3, THF, H_2O, 6 h; (iv) 2 N HCl, reflux, 1 h.

4f was purified as the *N*-BOC derivative **5f**. BOC cleavage and deprotection of the aminooxy groups afforded racemic

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