Synthesis and Biological Evaluation of a Series of 1,1-Dichloro-2,2,3-triarylcyclopropanes as Pure Antiestrogens

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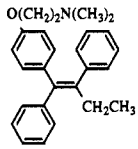
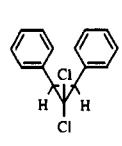
A series of 1,1-dichloro-2,2,3-triarylcyclopropanes (DTACs) was synthesized and evaluated as pure antiestrogens. Addition of 4-methoxy- or 4-(benzyloxy)phenyl Grignard reagents to *p*-methoxy, *p*-benzyloxy, or unsubstituted deoxybenzoins, followed by dehydration of the resulting carbinols produced a mixture of *E* and *Z* olefins, which were reacted with dichlorocarbene to give O-protected DTACs. The *E* and *Z* isomers were separated by fractional crystallization and the central or geminal phenyl ring was deprotected to provide phenolic DTACs. Alkylation with (*N*,*N*-dimethylamino)ethyl chloride yielded basic cyclopropanes. Two chlorodiarylindenes were isolated as thermolysis products of the DTACs, and one was converted to a phenol by hydrogenolysis. All DTACs and indenes were competitive inhibitors of [^{3}H]estradiol binding in the immature rat uterine cytosol receptor assay, with relative binding affinities of 0.1–3.6% of estradiol. None of the new compounds were estrogenic in the 3-day immature mouse uterotrophic assay at doses up to 750 μg. In the 3-day immature mouse antiuterotrophic assay, five DTACs with either a methoxy (**5a**), benzyloxy (**4d**, **5c**), or (dimethylamino)ethoxy (**7a**, **7b**) central ring side chain produced significant decreases in uterine weight at doses up to 750 μg. One compound, (*Z*)-1,1-dichloro-2-[4-[2-(dimethylamino)ethoxy]-phenyl]-2-(4-methoxyphenyl)-3-phenylcyclopropane (**7b**), elicited a dose-dependent decrease in vivo comparable to MER 25. These same five compounds, as well as the lead compound Analog II, were active in vitro against the estrogen-dependent MCF-7 human breast tumor cell line in a dose-dependent fashion.

Introduction

Antiestrogens block uterine growth and the growth of estrogen-dependent mammary tumors and are effective in the control of other diverse neoplastic diseases, as well as controlling and correcting various endocrine disorders. Their mode of action is not completely understood, but is known to include competitive inhibition at the estrogen receptor (ER),[1] as well as an estrogen irreversible cytotoxic action linked to their antagonism of calmodulin-activated cellular processes.[2] Tamoxifen (TAM, **1**), a clinically useful triarylethylene (TAE) antiestrogen, elicits varied estrogenic effects, including an increase in the incidence of hepatocellular carcinoma in rats at high doses[3] and a possible increased risk of endometrial carcinoma.[4] Besides TAM, other TAE antiestrogens also elicit mixed estrogen agonist–antagonist responses.[5] Incomplete remission of estrogen-dependent mammary tumors during treatment with the TAEs appears to be associated, at least in part, with the uterotrophic activity of these compounds.[6]



1, Tamoxifen



2, Analog II

Inhibition of estrogen is a potentially useful strategy for the treatment of hormone-dependent breast tumors in postmenopausal females and possible tumor prevention in premenopausal women. The purpose of this research was to search for antiestrogens that were devoid of estrogen agonist activity. Efforts in this area have led to several interesting experimental compounds. The triphenylethanol derivative MER 25 is a pure antiestrogen, but its clinical side effects preclude its use in humans.[7] The pure antiestrogenic activities of zindoxifene analogues[8] and the steroid ICI-164,384[9] have also been reported. Reports from our laboratory[10-13] and others[14,15] have demonstrated that the introduction of a dichlorocyclopropyl or dihydrocyclopropyl moiety in place of the olefinic link in estrogenic stilbenes greatly reduces or abolishes their estrogenic activity. One compound, Analog II (1,1-dichloro-2,3-*cis*-diphenylcyclopropane, **2**), has antiestrogenic properties with no estrogen agonist activity in the mouse, and is comparable to TAM against the hormone-dependent 7,12-dimethylbenz[*a*]anthracene-induced rat mammary tumor model.[16] For this work, **2** was chosen as the lead compound to design more potent cyclopropyl antiestrogens. Even though TAEs elicit undesired estrogen agonist activity, their utility as antiestrogens provided the basis for incorporating certain functional groups into **2**, such as a third phenyl ring and polar para substituents to yield 1,1-dichloro-2,2,3-triarylcyclopropanes (DTACs) resembling both **2** and the clinically useful TAEs. The 1,1-dichlorocyclopropyl and dihydrocyclopropyl derivatives of known estrogenic olefins[11-15,17] have a marked decrease in

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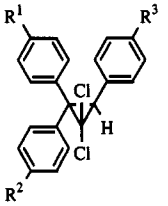
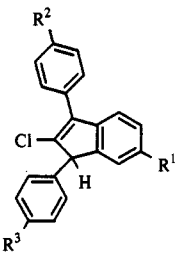
Table I. Yields and Physical Characteristics of 1,1-Dichloro-2,2,3-triarylcyclopropanes and 2-Chloro-1,3-diarylindenes

no.	R^1	R^2	R^3	method of prep	yield,[a] %	mp, °C	formula[b]
							
4a	H	H	H	A	64	106–107[c]	$C_{21}H_{16}Cl_2$[d]
4d[g]	OCH_2Ph	OCH_3	OCH_3	A	26	127–128[e]	$C_{30}H_{26}Cl_2O_2$[f]
5a	OCH_3	H	H	A	27	132–133.5[g]	$C_{22}H_{18}Cl_2O$[f]
5b	H	OCH_3	H	A	11	114.5–116.5[h]	$C_{22}H_{18}Cl_2O$[f]
5c	OCH_2Ph	OCH_3	H	A	32	173.5–175[i]	$C_{29}H_{24}Cl_2O_2$[f]
5d	OCH_3	OCH_2Ph	H	A	11	133–134[j]	$C_{29}H_{24}Cl_2O_2$[f]
6a	OH	H	H	B	84	135.5–136[k]	$C_{21}H_{16}Cl_2O$
6b	OH	OCH_3	H	C	99	oil	$C_{22}H_{18}Cl_2O_2$
6c	OCH_3	OH	H	C	99	133–135[l]	$C_{22}H_{18}Cl_2O_2$
7a	$O(CH_2)_2NMe_2$	H	H	D	5	140–142[m]	$C_{25}H_{25}Cl_2NO \cdot C_6H_8O_7$[n]
7b	$O(CH_2)_2NMe_2$	OCH_3	H	E	13	95.5–96.5[k]	$C_{26}H_{27}Cl_2NO_2$
8[g]	OH	OCH_3	OCH_3	C	95	96–97°	$C_{23}H_{20}Cl_2O_3$[f]
							
9a	OCH_3	OCH_2Ph	OCH_3	F	49	120–122[p]	$C_{30}H_{25}ClO_3$
9b	OCH_3	OCH_3	OCH_3	G	92	139–141[q]	$C_{24}H_{21}ClO_3$
10	OCH_3	OH	OCH_3	C	99	153–154[r]	$C_{23}H_{19}ClO_3$

[a] No attempts were made to optimize yields. [b] All compounds gave combustion elemental analyses that were within ±0.4% of theoretical values, except compound **8**, whose molecular weight was confirmed by low-resolution FAB-MS (see Experimental Section). [c] Petroleum ether. [d] See ref 19 (lit. mp = 105–107 °C). [e] Et_2O–petroleum ether. [f] Determined as a 1:1 mixture of *E* and *Z* isomers. [g] Me_2CO–petroleum ether. [h] SiO_2 chromatography. [i] Butanone then EtOAc. [j] Et_2O/EtOH. [k] EtOH. [l] Toluene–hexane. [m] EtOH/Et_2O. [n] Dihydrogen citrate salt. [o] C_6H_6–petroleum ether. [p] CH_2Cl_2–petroleum ether then recrystallized from boiling Et_2O. [q] 1:1 EtOH–Me_2CO. [r] Et_2O–hexane. [s] Mixture of *E* and *Z* isomers.

estrogenic activity. For example, the 1,1-dichlorocyclopropyl analogue of diethylstilbestrol has a relative uterotrophic potency 1.63% of estradiol in mice.[12] The 1,1-dichloro- rather than the dihydrocyclopropanes were studied in this project since the dihydrocyclopropyl analogue of TAM retains some of the estrogenic activity exhibited by the parent compound.[15]

This report describes the synthesis of DTACs bearing *p*-methoxy (**5a**), *p*-benzyloxy (**4d**, **5c**), and *p*-(dimethylamino)ethoxy (**7a**, **7b**) side chains on the central phenyl ring and their pure antiestrogenic activities in mice and in cell culture.

Chemistry

The compounds prepared in this study are listed in Table I. The general synthetic route to the DTACs (Scheme I) was the addition of *p*-methoxy- or *p*-benzyloxy-substituted Grignard reagents to *p*-methoxy or *p*-benzyloxy protected or unsubstituted deoxybenzoins (**2a–c**), followed by acid-catalyzed dehydration of the resulting carbinols to give a mixture of *E* and *Z* olefins (**3a–e**), which reacted with dichlorocarbene to give the fully protected DTACs **4a–e**. Fractional crystallization was utilized to separate **4b** and **4c** into their respective *E* and *Z* isomers, **5a–d**. Deprotection of the para substituent on the central or vicinal ring gave the phenolic cyclopropanes **6a–c**. Alkylation of the potassium salt of the central ring phenols **6a,b** with (dimethylamino)ethyl chloride yielded the basic DTACs **7a,b**.

Two methods of dichlorocarbene generation were utilized: the catalytic phase-transfer method[18–20] of 50% aqueous solution of NaOH, $CHCl_3$, and benzyltriethylammonium chloride (TEBA) as the anion-transfer agent; and the thermolysis of phenyl(bromodichloromethyl)mercury (Seyferth reageant).[21] Compound **4a** has been previously synthesized by the catalytic two-phase method,[19] and was prepared from the commercially available olefin **3a** in 64% yield.

Compound **5a** was fractionally crystallized from its *E* isomer **5b** with use of Me_2CO–petroleum ether, while **5b** was obtained as the more mobile isomer from the mother liquors by preparative TLC. The 60-MHz NMR spectrum of **5a** had signals for the ring protons ortho to the methoxy group 0.2 ppm upfield to those of **5b**. The remaining aromatic signal for **5a** was more complex than that of **5b**, which gave a singlet for 10 protons. Proton NMR data of the TAEs show that the central ring signals appear at higher field than those of the vicinal and geminal rings due to the double shielding effect of the flanking rings' current.[22] These NMR isomer assignments are supported by the X-ray crystallographic studies of the *Z*[23] and *E* iso-

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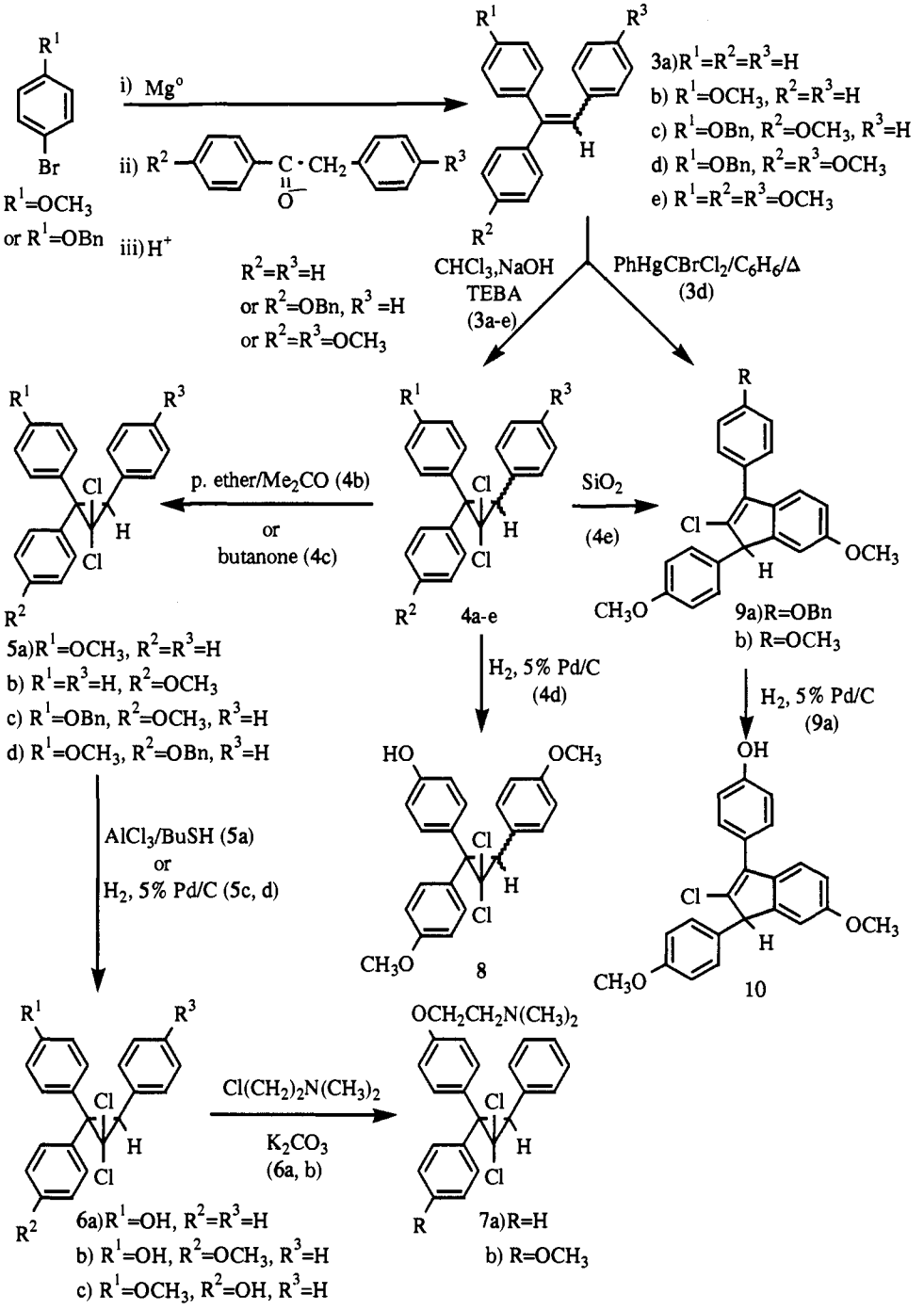
Scheme I



mers[24] of TAM, which show that the 1,1,2-triphenyl ring system adopts a pinwheel- or propeller-like arrangement to maximize intramolecular atomic separation between the phenyl rings. By comparison of molecular models, it is apparent that the same paramagnetic influences seen in the TAEs are operative in the DTACs.

Phenol **6a** are synthesized from methyl ether **5a** by using the hard acid–soft base ($AlCl_3$–BuSH) method[25] in yields of 80–100%. Of the available methods for aryl ether demethylation,[26] only the Lewis acids were considered safe to the cyclopropyl ring and retention of both of the chlorine atoms. The commonly used Lewis acid BBr_3[27-29] was of unreliable utility for aryl ether demethylation of the DTACs in our hands.

The dichlorocyclopropyl analogue of TAM, compound **7a**, was obtained as the citrate salt in low yield (5%) by alkylation of **6a** with (*N*,*N*-dimethylamino)ethyl chloride hydrochloride and excess K_2CO_3 in refluxing Me_2CO, followed by chromatographic purification and salt formation with anhydrous citric acid.

Phase-transfer dichlorocyclopropanation of **3c**, followed by trituration of the crude reaction mixture with boiling butanone yielded the *Z* isomer **5c** in 32% yield. *E* isomer **5d** was obtained in 11% yield after flash chromatography of the mother liquor and crystallization from EtOH. Higher field proton NMR (300 MHz) spectra were required

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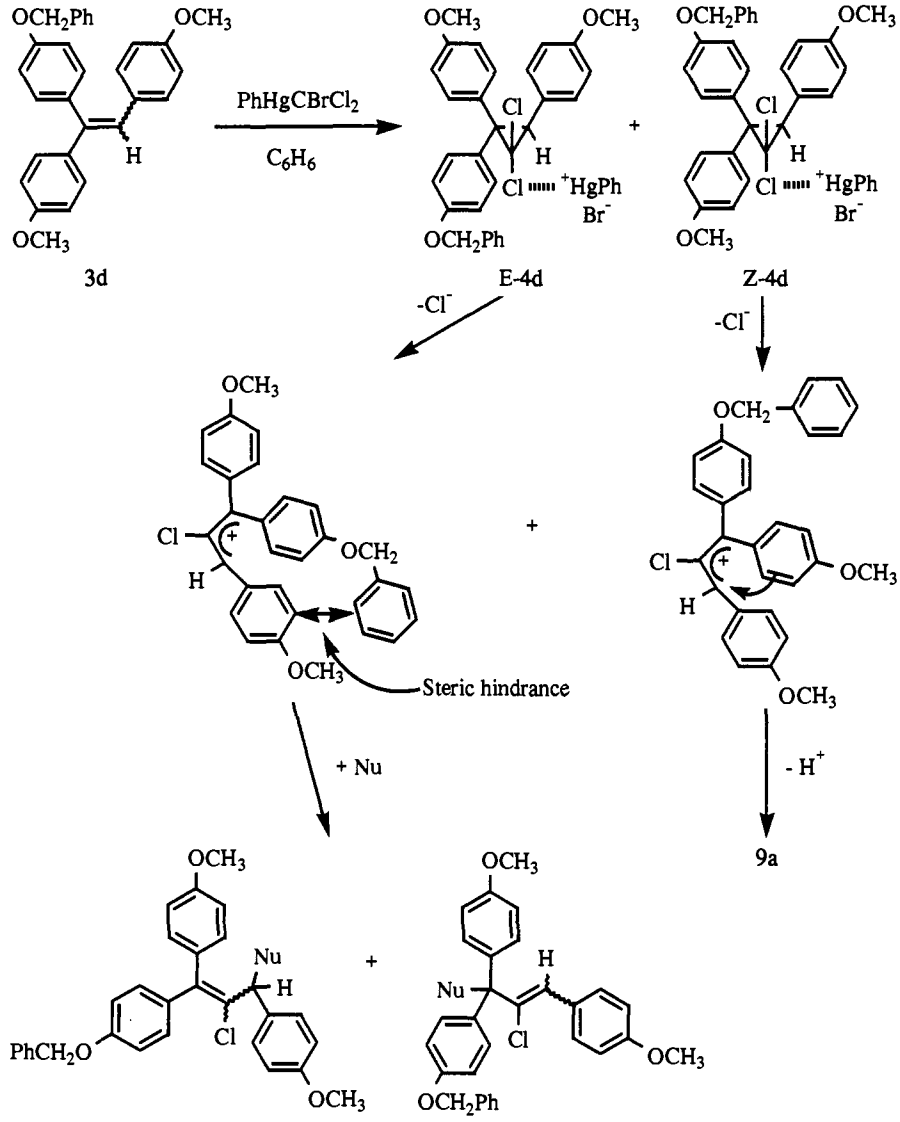
Scheme II



to determine the shifts of these isomers since, at 60 MHz, the aromatic regions were too complex for accurate interpretation and signals for the benzyl methylene and the methoxy groups each appeared as unresolved singlets. The 300-MHz benzyl methylene signal of *Z* isomer **5c** was 0.05 ppm upfield to that of *E* isomer **5d**.

The (*Z*)-benzyl ether **5c** and the (*E*)-benzyl ether **5d** gave the (*E*)-phenol **6b** and the (*Z*)-phenol **6c**, respectively, in nearly quantitative yields after hydrogenolysis of the benzyl protecting group with 5% Pd/C at room temperature and atmospheric pressure. Selective hydrogenolysis of the benzyl protecting group in the presence of the cyclopropyl ring apparently takes place due to the ability of the bulky *gem*-dichloro group to interfere with chemisorption of the cyclopropyl ring to the catalytic surface.[30] The isolated methylene ^{1}H NMR signal of the benzyl group, along with the ease with which it can be removed and leave the dichlorocyclopropyl system intact, made this the method of choice for protecting phenolic functions in dichlorocyclopropanes.

The (*Z*)-anisyldichlorocyclopropyl analogue of TAM (**7b**) was isolated in low yield (13%) after the alkylation of phenol **6b** in a modification of the method used for synthesis of **7a**, where a 4-fold excess of (dimethylamino)ethyl chloride was used. Both the hydrochloride and citrate salts of **7b** were hygroscopic, and hence, **7b** was isolated only as the free base after repeated chromatographic purification on SiO_2.

Phase-transfer cyclopropanation of trimethoxy olefin **3e** gave **4e** in crude form in 92% yield. Subjection of **4e** to SiO_2 flash chromatographic purification resulted in rearrangement to the 2-chlorodiarylindene (CDI) **9b**. The thermal loss of HCl with rearrangement to give 2-chloro-1,3-diphenylindene has been reported to occur following attempts to purify **4a** by sublimation.[19] Also, the thermal rearrangement of (*E*)-1,1-dichloro-2,3-diphenylcyclopropane to 2-chloro-1-phenylindene at sublimation conditions was reported earlier by our laboratory.[31] Based on much literature precedence,[32-35] the proposed mechanism for the thermal loss of HCl and the rearrangement of the 1,1-dichlorocyclopropanes is via concerted cleavage of the C^2–C^3 σ bond of the cyclopropyl group with a shift of the uncoupled electrons' orbitals in a direction that

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places them anti to the chloride leaving group to give S_N2 displacement in concert with the formation of an allyl cation. Two alternative disrotatory processes are possible. However, an inspection of molecular models of the DTACs illustrates that the disrotatory process that involves the turning of the uncoupled orbitals toward the face of cyclopropane originally containing the geminal ring is physically impossible, since the central and vicinal rings would collide. After allyl cation formation, the original geminal ring is placed in close proximity to cyclopropyl C^3, where the ring's π electrons attack to form an indenyl cation, which subsequently loses a proton to complete aromatization of the phenyl portion of the indene ring system.

Differential substitution of the tri-para-substituted DTACs was originally attempted by way of reaction of olefins **3d** with the dichlorocarbene generated from the Seyferth reagent. However, this reaction resulted in formation of indene **9a** in 49% yield, via the loss of HCl and rearrangement of the *Z* isomer of intermediate **4d**. The mechanism of dichlorocarbene generation from phenyl(bromodichloromethyl)mercury involves the concerted extrusion of the dichloromethylene and formation of phenylmercuric bromide. Since the phenylmercuric cation is a Lewis acid, its coordination with the chloride leaving from the cyclopropane could assist in the rearrangement of **4d**. Another possible contributing factor to the complete loss of cyclopropane from the reaction mixture is that the temperature required for thermolysis of the Seyferth reagent in C_6H_6 is 88 °C, which may be higher than that required to induce thermolysis of **4d** in C_6H_6. High-field proton NMR of **9a** displayed one singlet for the benzyl methylene signal and two singlets for the methoxy signals. The 75.5-MHz ^{13}C NMR spectrum of **9a**, as well as capillary GC analysis, confirmed the presence of only one isomer. It is proposed that only one indene is formed due to steric restraints caused by the bulky geminal ring benzyloxy group (see Scheme II) in the allyl cation formed from the *E* isomer of **4d**.

Indene **9a** was debenzylated by hydrogenolysis to give phenol **10** in 99% yield. Attempts at addition of the (dimethylamino)ethoxy side chain to **10** at 40 °C in Me_2CO–EtOH with K_2CO_3 as base resulted in a product with spectral characteristics that suggested the relatively acidic benzyl proton of **10** had, in addition to the phenolic H, been removed, with further reaction on the cyclopentadienyl group, possibly with solvent,[36] although the structures of the reaction products have yet to be elucidated.

Phase-transfer cyclopropanation of olefins **3d** gave cyclopropanes **4d** in 26% yield. Purification of **4d** proved to be a formidable task. The compound decomposed in solution on contact with sand, SiO_2, C_{18} reverse-phase packing, scratched, sintered, and ground glass, at relatively low temperatures (boiling EtOH and 2-PrOH), and in some polar aprotic solvents (DMSO, slowly; DMF, rapidly). Fortunately, **4d** was stable in solution to contact with glass wool, and rapid preparative column chromatography over neutral Al_2O_3 using C_6H_6–petroleum ether as eluents allowed enough purification for crystallization of pure **4d**. The added stabilization of the intermediate allyl cation formed from the DTACs with para-directing substituents on all three phenyl rings requires that great care be used in their isolation.

The mixture of (*E*)- and (*Z*)-**4d** was debenzylated by hydrogenolysis to give phenols **8** in 95% yield. The first solid obtained from the reaction tenaciously held Et_2O (1.8 mol/mol of **8** as determined by NMR), even after extended attempts at ether removal under vacuum. Several recrystallizations yielded a white powder that showed no Et_2O by NMR, but failed to yield a satisfactory combustion

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Table II. Estrogen Receptor Binding Affinities[a] and Antiuterotrophic Activities[b] of 1,1-Dichloro-2,2,3-triarylcyclopropanes and 2-Chloro-1,3-diarylindenes

no.	RBA,[c] %	dose, μg	antiuterotrophic % reduction[d] (±SEM)
4a	0.1	30	11 ± 1
		150	3 ± 8
		750	13 ± 8
4d[e]	0.1	10	2 ± 7
		30	15 ± 14
		150	49 ± 14***[g]
		750	2 ± 9
5a	0.2	30	22 ± 6
		150	49 ± 9**
		750	33 ± 8*
5c	0.1	30	37 ± 10*
		150	47 ± 11**
		750	22 ± 6
6a	0.7	30	23 ± 8
		150	12 ± 16
		750	25 ± 11
6b	1.7	30	15 ± 5
		150	−7 ± 10
		750	41 ± 8***
6c	2.4	30	28 ± 10
		150	18 ± 8
		750	22 ± 6
7a[f]	0.9	4	−6 ± 6
		10	10 ± 8
		30	37 ± 9*
		150	30 ± 10
		750	18 ± 4
7b	0.7	4	−10 ± 6
		10	6 ± 7
		30	33 ± 8*
		150	42 ± 10*
		750	53 ± 11**
8[e]	3.6	30	19 ± 12
		150	10 ± 9
		750	26 ± 6*
9a	0.1	30	14 ± 13
		150	4 ± 10
		750	0 ± 13
9b	0.1	30	27 ± 10*
		150	−6 ± 10
		750	28 ± 20
10	0.5	30	3 ± 7
		150	14 ± 12
		750	12 ± 20
tamoxifen	0.9	30	4 ± 9
		150	0 ± 10
		750	−12 ± 5
MER 25	0.002	30	1 ± 13
		150	30 ± 6*
		750	82 ± 6****
Analog II	0.009	200	28 ± 4*
		400	42 ± 4***
		800	58 ± 9****
estradiol	100		

[a] Determined by competitive radiometric binding assay with rat uterine cytosol as a source of receptor, [^{3}H]estradiol as tracer, and dextran-coated charcoal as absorbant for free ligand. [b] Determined as the decrease in the estradiol-stimulated (0.03 μg total dose) uterine weight of immature (17–19 days old) female mice. [c] Binding affinities are expressed relative to that of estradiol = 100% (RBA = relative binding affinity) and are the average of duplicate determinations minus nonspecific binding. [d] Calculated by 100 − {(mean uterine weight of test compound treated animals − mean uterine weight of control animals)/(mean uterine weight of estradiol-stimulated animals − mean uterine weight of controls)} × 100. There were no significant differences in final body weights between test groups. [e] Determined as a 1:1 mixture of isomers. [f] Dihydrogen citrate salt. [g] (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.005$, (****) $p < 0.001$.

Table III. Effect of 1,1-Dichloro-2,2,3-triarylcyclopropanes, TAM, and Analog II on the Growth of Estrogen Receptor Positive MCF-7 Human Breast Cancer Cell Line

	% inhibn of control cell growth ± SEM[a] conen, M		
compound	10^{-9}	10^{-7}	10^{-5}
TAM	20.5 ± 8.9[b]	51.8 ± 2.4[b]	71.9 ± 1.5[b]
Analog II	52.1 ± 4.0[b]	60.4 ± 1.6[b,c]	81.3 ± 0.3[b]
4d	15.8 ± 0.5[b]	46.2 ± 3.8[b]	90.9 ± 0.2[b]
5a	6.1 ± 5.5	35.4 ± 1.8[b]	70.9 ± 2.9[b]
5c	31.3 ± 4.9[b]	34.7 ± 1.7[b]	48.5 ± 10.3[b]
7a[d]	12.4 ± 5.9	41.4 ± 4.5[b]	96.3 ± 0.5[b]
7b	26.9 ± 7.5[b]	35.1 ± 2.2[b]	63.5 ± 2.0[b]

[a] Mean of triplicate observations. [b] One-way ANOVA indicated significant inhibition of cell growth ($p < 0.05$). Duncan's new multiple range test was used to compare individual groups. [c] Tested at a concentration of 10^{-6} M. [d] Dihydrogen citrate salt.

elemental analysis. The molecular weight of 8 was confirmed by low-resolution FAB-MS.

Phenol 8 rapidly rearranged with the loss of HCl when dissolved in dry DMF. Compound 8 also slowly decomposed in DMSO-d_6 (50 mg in 0.5 mL, NMR tube, 15 min) to give a complex mixture of products. It is proposed that these polar aprotic solvents, known to be excellent milieus for ionic intermediates, allowed the solvolytic loss of chloride and simultaneous rupture of the C^2–C^3 bond of the cyclopropyl ring. Attempts to alkylate 8 with (dimethylamino)ethyl chloride and K_2CO_3 in refluxing Me_2CO, and also at 40 °C in 1:1 EtOH–Me_2CO, gave only ring-opened products. Alkylation at room temperature in the latter solvent system yielded the desired crude product, but attempts at purification (SiO_2 using 19:1 C_6H_6–Et_3N or Me_2CO; basic Al_2O_3 using 1:1 $CHCl_3$–Et_2O or CH_2Cl_2; sublimation; simple acid–base extraction methods) resulted in intractable mixtures of ring-opened products.

Biological Evaluation

The biological evaluation of the test compounds are summarized in Tables II and III and consisted of the in vitro rat cytosolic estradiol receptor binding assay, the in vivo immature mouse uterotrophic (estrogenic) assay, the in vivo immature mouse antiuterotrophic (antiestrogenic) assay, and the in vitro suppression of the proliferation of the ER positive MCF-7 human breast cancer cell line. Estradiol (pure agonist), TAM (agonist in mice, antagonist in MCF-7), MER 25 (pure antagonist), and Analog II (antagonist in mice) were used in the assays as standards.

Receptor-Binding Assay. The relative binding affinities (RBAs), as determined by the competitive radiometric binding assay and measured by displacement of [^{3}H]estradiol from rat uterine cytosol receptor preparation, after subtracting nonspecific receptor/protein binding, were calculated for all of the synthesized target compounds, TAM, MER 25, Analog II, and estradiol. All of the new compounds showed some degree of concentration-dependent displacement of radiolabel from the ER (Table II), with RBAs ranging from 0.1 to 3.6% of that of estradiol, with TAM yielding a RBA value of 0.9%. Phenolic compounds yielded the highest activities (**8** > **6c** > **6b** > **6a** > **10**), ranging from 3.6 to 0.5%. The two (dimethylamino)ethoxy side-chain-bearing compounds gave RBAs similar to TAM with compound **7a** showing an affinity equivalent to TAM (0.9%). The RBA of compound **7b** was slightly lower (0.7%). The unsubstituted compound **4a**, as well as the fully O-protected DTACs (**5a**, **5c**, and **4d**) and CDIs (**9a** and **9b**) had much lower RBAs, ranging from 0.2 (**5a**) to 0.1% (**4d**, **9a**).

Uterotrophic Assay. The test compounds showed no uterotrophic activity when tested in vivo in the absence of estradiol at doses to 750 μg (data now shown). TAM elicited a significant estrogenic response at a total dose of 1 μg, while MER 25 was slightly estrogenic only at the high total dose of 750 μg. Analog II was not estrogenic at doses up to 1000 μg.

Antiuterotrophic Assay. All compounds were tested for their ability to antagonize the uterine weight gain in immature mice from a stimulating dose (0.03 μg) of estradiol at doses of 30, 150, and 750 μg. Compounds **4d**, **5c**, **7a**, and **7b** were tested at total doses as low as 4 μg to define their lower limits of antiuterotrophic activity. Values obtained at the various dose levels are summarized in Table II. None of the compounds potentiated the uterotrophic action of estradiol at the doses tested. Compounds **4d**, **5a**, **5c**, and **7a**,**b** produced significant decreases in uterine weight, with **7b** exhibiting a dose-dependent decrease. As expected, TAM had no antiestrogenic activity in the mouse uterus at any of the doses tested, while both MER 25 and Analog II yielded a dose-dependent decrease in uterine weight.

MCF-7 Cell Culture Assay. Five of the DTACs (**4d**, **5a**, **5c**, **7a**, and **7b**), Analog II, and TAM were tested for their antiproliferative activity in the MCF-7 human breast cancer cell line (Table III). Compounds were tested at 10^{-5} to 10^{-9} M for their ability to alter the growth of cells. All of the test compounds were active against the ER positive MCF-7 cell line in a dose-dependent fashion.

Results and Discussion

None of the test compounds exhibited estrogenic activity, yet were competitive ligands for the ER, albeit weakly so. All of the DTAC receptor binding affinities were in the range of 11–400 times higher than that of Analog II, due, at least in part, to increased hydrophobic interactions with the ER caused by the additional phenyl group. A *p*-hydroxy group on the geminal ring gave DTACs with higher RBAs than those with a similar substitution on the central ring. This is in agreement with a previous report[37] that a phenolic group on the geminal ring is the most important requirement in TAEs for ER affinity.

Compounds **4d** and **8** contain a tri-para-substituted DTAC nucleus. Since the potent antiestrogen H-1285 is tri-para-substituted (i.e., a vicinal ring methoxy–geminal ring hydroxy derivative of TAM) and possesses very high affinity for the ER,[38] it was of interest to incorporate as many of the structural attributes of this TAE into a DTAC nucleus. Interestingly, although **8** was the highest affinity DTAC or CDI ligand for the ER studied, it elicited no estrogenic activity. Compound **4d** was a poor ER ligand, yet was potently antiuterotrophic and antiproliferative.

Compound **7a** is the 1,1-dichlorocyclopropyl analogue of TAM, and was designed to determine if the 1,1-dichlorocyclopropyl group in place of TAM's butenyl group would confer pure antiestrogenic properties on the resulting compound. Compounds **7a** and **7b** had RBAs identical with that of TAM, but were not uterotrophic in mice. The antiuterotrophic effect of **7b** was dose-dependent in vivo, the only DTAC with this property. Both **7a** and **7b** were antiproliferative in the MCF-7 cell culture assay in a concentration-dependent manner. Compound **7a** had the best antiproliferative profile of all the test compounds. This compound's poor antiuterotrophic activity in vivo was possibly a function of absorption from the site of injection, as **7a** was tested as its citrate salt. The apparent lack of estrogenic activity of **7a** and **7b** is in-

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teresting in comparison to the report of Bedford et al.,[15] that the dihydrocyclopropyl analogue of TAM had weak estrogenic activity in combination with an antiestrogenic activity (in the rat) 167-fold weaker than that of TAM. Also, the potent estrogenic activity seen in mice with TAM is completely absent from compounds **7a** and **7b**, obviously due to the introduction of the 1,1-dichlorocyclopropyl group in place of the olefinic butenyl group.

One of the most intriguing results of this work is the activity profiles seen with the *O*-benzyl protected cyclopropanes **4d** and **5c**, as well as the methoxylated central ring compound **5a**. Even though the *p*-benzyloxy substituent on the central ring is relatively nonpolar, it is electron rich at its distal region (as are the (dialkylamino)ethoxy and 2,3-dihydroxybutoxy substituents in known TAEs). A recent report shows that this substituent confers partial estrogen agonist activity on triphenylbutenes in cell culture,[39] and a communication relating the effect of this substituent on fertility in a short series of di- and triaryldihydrocyclopropanes has been published.[40] Our incorporation of the dichlorocyclopropyl moiety apparently transforms these weak estrogens to antagonists.

The antiestrogenic activities seen with **4d**, **5a**, and **5c** support the finding that a (dialkylamino)ethoxy side chain is not required for antagonism of estradiol-induced uterine weight gain. A nonpolar substituent at the para position of the central ring may still elicit antagonist activity, as evidenced by the antiestrogenic activity of the central ring ethylated TAE broparestrol.[41] Also, in vitro ER affinity is not an accurate gauge of the possible in vivo antiestrogenic activity of a compound, as metabolic activation in vivo to a more potent antiestrogen (e.g. TAM to 4-hydroxyTAM) can occur. The antiestrogenic compounds **4d**, **5a**, and **5c** were poor ligands in the binding assay. Removal of the benzyl groups from these compounds to yield the corresponding phenols (**8**, **6a–b**) resulted in a complete loss of antiestrogenic activity.

The CDIs **9a,b** and **10** were poorer ligands for the ER than their corresponding DTACs. However, the same structural requirements for increasing ER affinity in the cyclopropanes are applicable in the indenes in that a benzyl or methyl ether reduces affinity 5-fold compared to a phenolic group. Molecular models of the previously unknown CDIs show significant structural similarities to those of the TAEs (data not shown). When the phenyl portion of the indene ring system is superimposed on the central ring of a TAE, the 1- and 3-rings of the indene partially superimpose with the geminal and vicinal rings of the TAEs. The lack of activity of the CDIs in both the uterotrophic and antiuterotrophic assays suggests that the functional groups important for activity in the DTACs are not situated in the proper position in space in the CDIs to effect the same activity. The phenyl groups of the CDIs have more rotational freedom than the TAEs and may not adopt the same propeller-like conformation. The resulting lack of conformational interdependence of the phenyl groups on the 1,3-diarylindenes could be a contributing factor to their reduced activity.

The significance of this work is that several of the 1,1-dichloro-2,2,3-triarylcyclopropanes (**4d**, **5a**, **5c**, **7a**, and **7b**) were found to be structurally distinct antiestrogens devoid of estrogenic activity in the mouse. They also inhibited the growth of ER positive MCF-7 cells in a dose-dependent manner, as did the lead compound Analog II.

In the DTACs studied, it is apparent that a methoxy group at the para position of either the geminal or vicinal rings is important for antiestrogenic activity, although the position in space that this group should occupy in relation to the receptor is not easily discerned. It is likely that a methoxy substituent at the para position of the geminal ring of the DTACs mimicking the C^3-phenolic site of estradiol is important for antiestrogenic activity. Compound **5a**, with its methoxy group at the para position of the central ring, may be oriented at the receptor in such a manner that its methoxy group occupies the same region that this group in compounds **4d**, **5c**, and **7b** does, with the remainder of the structure of compound **5a** perturbing its interaction with the ER to an extent that antagonism is its mode of action. It was found that the antiestrogenic action of **5c** is not due to its rearranged indene product **9a**, which did not elicit an antiuterotrophic response.

The finding that compounds **4d** and **5c** are antiestrogenic adds the benzyloxy group to the list of side chains, already known to include (dialkylamino)ethoxy, 2,3-dihydroxybutoxy, and ethyl, that may elicit an antiestrogenic response when on the para position of the central ring of a compound containing the 1,1,2-triaryletha(e)ne nucleus.[42] The bulky benzyl group may prevent the activation of the receptor by sterically blocking the conformational change required after receptor binding, much as the (dialkylamino)ethoxy side chain has been hypothesized to perturb the activation of the ER.[42] In light of recent evidence,[39] a dichlorocyclopropyl group is apparently an important requirement for pure antagonism, also. Compound **7b**, the dichlorocyclopropyl geminal ring anisyl analogue of TAM, was antiuterotrophic in mice in vivo and antiproliferative in MCF-7 cells in vitro in a dose-dependent manner. In stark contrast to TAM, **7b** had no in vivo estrogen agonist activity. The pure antiestrogenic activities of **4d**, **5a**, **5c**, **7a**, and **7b**, as well as the lead compound Analog II, are presently under further mechanistic study.

Experimental Section

The structures of all compounds were supported by their proton NMR spectra, which were measured on either a Varian EM-360A or a XL-300 spectrometer. The spectra are reported in parts per million in $CDCl_3$ with tetramethylsilane as the internal standard. ^{13}C NMR were determined on the XL-300 at 75.5 MHz, referenced by the $CDCl_3$ signal. Gas chromatography-mass spectral analyses were performed in the EI mode on a Hewlett-Packard 5995 GC–MS system using a 30 m × 0.25 mm DB-1 fused silica capillary column (J and W Scientific). Positive ion FAB-MS were determined on a VG analytical ZAB-E spectrometer at 0–25 °C in a 3-nitrobenzyl alcohol matrix ionized at 0.95–2 V with the source operated at 8 kV using xenon as the discharge gas. High-resolution spectra were calibrated with polyethylene glycol. Infrared spectra were obtained from KBr pellets on a Beckman Acculab 1 spectrometer and were consistent with the assigned structures. Silica gel (J. T. Baker) of approximately 40-μm diameter was used for flash chromatography,[43] which was performed at 5–10 psi. Petroleum ether was of bp 30–60 °C. When necessary, solvents or reagents were dried by appropriate methods. Evaporations were carried out in vacuo on a rotary evaporator or under a stream of dry N_2. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Reaction progress and product purity were monitored by analytical TLC on strips of Eastman Kodak plastic-backed SiO_2 60 F_{254} or Al_2O_3 F_{254}. Developed strips were viewed under light of 254- and 365-nm wavelengths. Elemental analyses were done by Midwest Microlab Ltd., Indianapolis, IN. Analytical results were within ±0.4% of theoretical values. Acceptable elemental results are denoted in Table I by the formula, followed by the elements analyzed. Melting points and yields of the DTACs and CDIs, based on pure

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samples unless otherwise noted, are listed in Table I.

The deoxybenzoins, 4-bromoanisole, and triphenylethylene **3a** were purchased from Aldrich Chemical Co. Olefins **3b** and **3e**,[44] 4-(benzyloxy)bromobenzene,[45] and 4-(benzyloxy)phenyl benzyl ketone[46] were prepared by standard methods.

1-[4-(Benzyloxy)phenyl]-1-(4-methoxyphenyl)-2-phenylethene (3c). To the Grignard reagent prepared from Mg turnings (1.52 g, 63 mg-atom) and 4-bromoanisole (13.45 g, 9 mL, 72 mmol; initiated with one I_2 crystal and one drop $EtBr_2$) in 25 mL of THF was added 4-(benzyloxy)phenyl benzyl ketone[46] (3 g, 9.9 mmol). The reaction mixture was stirred at reflux for 48 h and then treated with 10 mL of saturated aqueous NH_4Cl. After cooling to room temperature, the slurry was filtered, the filter cake was washed with 50 mL of THF, and the filtrates were concentrated to give an orange oil. The oil was chromatographed over 60 g of flash SiO_2 (petroleum ether followed by Me_2CO). The Me_2CO fractions were combined, concentrated, dissolved in 100 mL of 1:1 95% EtOH–2 N H_2SO_4, and heated to reflux with stirring 8 h. The solvents were removed to give a brown gum, which was dissolved in 100 mL of Et_2O, washed with saturated aqueous $NaHCO_3$ (100 mL), brine (100 mL), and H_2O (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a brown oil. The oil was purified on 60 g of flash SiO_2 (9:1 petroleum ether–CH_2Cl_2) to give 3.1 g of **3c** (80%) as a clear oil: NMR δ 7.41 (br s, 5 H, *O*-benzyl C_6H_5), 7.3–6.75 (m, 9 H, substituted rings' ArH and C=CH), 7.11 (s, 5 H, $=CC_6H_5$), 5.05 (s, 2 H, OCH_2Ph), 3.79 (s, 3 H, OCH_3).

1-[4-(Benzyloxy)phenyl]-1,2-bis(4-methoxyphenyl)ethene (3d). 4-(Benzyloxy)bromobenzene (13.155 g, 50 mmol) in 20 mL of THF was added to Mg turnings (1.17 g, 48 mg-atom) in 20 mL of THF. Grignard reagent formation was facilitated by addition of a few drops of $EtBr_2$, one crystal of I_2, and heat. This mixture was stirred and heated to reflux for 2 h. Desoxyanisoin (11.54 g, 45 mmol) was added under reflux as a slurry in 60 mL of THF. The resulting mixture was stirred at reflux for 18 h, cooled to room temperature, poured onto 200 g of 2 N H_2SO_4 and ice (1:1), and stirred at room temperature until the ice melted. The resulting mixture was extracted with 200 mL of Et_2O. The organic layer was washed with aqueous Na_2SO_5 (100 mL) and brine (2 × 100 mL). The combined aqueous layers were extracted with Et_2O (50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give 19 g of a wine-colored oil, which was dissolved in Et_2O and refrigerated to precipitate any unreacted desoxyanisoin. After filtration and concentration of the solution, the resulting oil was purified on 60 g of flash SiO_2 (7:3 petroleum ether–CH_2Cl_2) to yield 12.4 g (65%) of **3d** as a yellow oil: NMR δ 7.41 (s, 5 H, *O*-benzyl C_6H_5), 7.35–6.57 (m, 13 H, ArH and C=CH), 5.07 (s, 2 H, $PhCH_2O$), 3.78 (s, 1.5 H, geminal ring OCH_3), 3.77 (s, 1.5 H, central ring OCH_3), 3.72 (s, 3 H, vicinal ring OCH_3).

Method A. (*E*)- and (*Z*)-1,1-Dichloro-2-[4-(benzyloxy)phenyl]-2,3-bis(4-methoxyphenyl)cyclopropane (4d). Olefin **3d** (20 g, 47.4 mmol) and 1 g of TEBA (4.39 mmol) in 100 mL of $CHCl_3$ were treated with 80 mL of chilled 50% NaOH with rapid stirring for 30 h. The dark emulsion was poured into a separatory funnel containing 100 mL of H_2O and 100 mL of CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with H_2O (3 × 100 mL), dried (K_2CO_3), filtered, and concentrated to yield a dark oil, which was dissolved in C_6H_6 and chromatographed on 200 g of Al_2O_3 (activity I, 1:1 C_6H_6–petroleum ether) at 5 psi. Fractions 2–6 (100 mL each) were concentrated to give an orange oil, which was dissolved in 200 mL of petroleum ether containing ca. 10 mL of Et_2O. After standing at room temperature 4 days **4d** precipitated as a white powder in four crops: NMR δ 7.39 and 6.82 (AA'BB', 4 H, J = 8.5 Hz, ArH), 7.36–7.25 (m, 5 H, C_6H_5), 7.19 and 6.80 (AA'BB', 4 H, J = 8.5 Hz, ArH), 6.91 and 6.74 (AA'BB', 4 H, J = 8.5 Hz, ArH), 4.92 (s, 2 H, OCH_2Ph), 3.70 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 3.47 (s, 1 H, cyclopropyl H). This compound readily decomposes in solution with evolution of HCl gas on contact with scratched, sintered, or ground glass.

1,1-Dichloro-2,2,3-triphenylcyclopropane (4a) was prepared as previously described:[19] NMR δ 7.23 (m, 15 H, ArH), 3.57 (s, 1 H, cyclopropyl H); MS m/z (% base) 342 (M + 4, 3.2), 340 (M + 2, 6.3), 338 (M^+, 10.23), 303 (–Cl, 100), 267 (–Cl, –HCl, 80.7).

(*Z*)-1,1-Dichloro-2,3-diphenyl-2-(4-methoxyphenyl)cyclopropane (5a) and *E* Isomer 5b. Phase-transfer reaction of *E* and *Z* olefins **3b** (5 g, 17.5 mmol) gave a white powder in 38% yield after flash chromatography (4:1 petroleum ether–CH_2Cl_2) and recrystallization from petroleum ether or pentane (mixture of **5a** and **5b**): mp 118–123 °C; NMR δ 7.15 (m, 14 H, ArH), 3.76 (s, 3 H, OCH_3), 3.52 (s, 1 H, cyclopropyl H); MS m/z (% base) 334 (–Cl, 7.09), 297 (–Cl, –HCl, 100). The *Z* isomer **5a** was isolated by fractional crystallization: NMR δ 7.33 (s, 10 H, ArH), 6.72 and 7.52 (AA'BB', 4 H, central ring), 3.71 (s, 3 H, OCH_3), 3.52 (s, 1 H, cyclopropyl H). The *E* isomer **5b** was obtained as clear crystals by thin-layer chromatography (SiO_2, petroleum ether) as the more mobile compound: NMR δ 7.33 (s, 10 H, ArH), 6.92 and 7.33 (AA'BB', 4 H, geminal ring), 3.79 (s, 3 H, OCH_3), 3.52 (s, 1 H, cyclopropyl H).

(*Z*)-1,1-Dichloro-2-[4-(benzyloxy)phenyl]-2-(4-methoxyphenyl)-3-phenylcyclopropane (5c) and *E* Isomer 5d. The oil from the phase-transfer reaction of olefin **3c** (6 g, 15.3 mmol) was treated with Et_2O–petroleum ether to give a 1:1 mixture of **4c**: mp 129–134 °C. The *Z* isomer **5c** precipitated as a white powder in two crops and was recrystallized: NMR δ 7.42–7.17 (m, 12 H, ArH), 7.01 (m, 2 H, ArH), 6.84 (d, 4 H, J = 8.7 Hz, ortho signals), 4.98 (s, 2 H, OCH_2), 3.75 (s, 3 H, OCH_3), 3.5 (s, 1 H, cyclopropyl H). The *E* isomer **5d** was obtained by concentration of the mother liquor and purification over flash SiO_2 (4:1 petroleum ether–CH_2Cl_2), followed by recrystallization: NMR δ 7.43–6.95 (m, 9 H, ArH), 7.36 (s, 5 H, *O*-benzyl C_6H_5), 7.1 (d, 2 H, J = 8.7 Hz, meta signals), 6.78 (d, 2 H, J = 8.7 Hz, ortho signals), 5.03 (s, 2 H, OCH_2), 3.73 (s, 3 H, OCH_3), 3.5 (s, 1 H, cyclopropyl H).

Method B. (*Z*)-1,1-Dichloro-2,3-diphenyl-2-(4-hydroxyphenyl)cyclopropane (6a). To cooled *n*-BuSH (0.63 g, 7 mmol) in 6 mL of CH_2Cl_2 was added $AlCl_3$ (0.75 g, 6 mmol) under Ar. Methyl ether **5a** (0.5 g, 1.35 mmol) in 15 mL of CH_2Cl_2 was added dropwise at 0 °C. After the mixture was stirred 2 h at room temperature, the reaction was quenched with 20 mL of H_2O. The yellow mixture was extracted with Et_2O (2 × 40 mL). The combined organic layers were washed with 30 mL of 0.1 N H_2SO_4, dried (Na_2SO_4), and concentrated to give 475 mg of an amber oil. Flash chromatography on 20 g of SiO_2 (1:1 CH_2Cl_2–petroleum ether) yielded an off-white glass. Crystallization yielded 400 mg of **6a** as white needles: NMR δ 7.38 and 6.58 (AA'BB', 4 H, central ring), 7.02 (m, 10 H, ArH), 4.75 (br s, 1 H, OH), 3.53 (s, 1 H, cyclopropyl H); MS m/z (% base) 320 (–Cl, 6.96), 319 (–HCl, 6.03), 318 (–H, –HCl, 27.05), 283 (–2HCl, 100).

Method C. (*E*)-1,1-Dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)-3-phenylcyclopropane (6b). Benzyl ether **5c** (1 g, 2.1 mmol) was dissolved in 50 mL of dry THF and hydrogenolyzed with H_2 in the presence of 50 mg of 5% Pd/C at 1 atm and room temperature for 8 h. The resulting clear solution was filtered, concentrated to give a red oil, and purified on 20 g of flash SiO_2 (1:1 petroleum ether–CH_2Cl_2) to yield 0.8 g of **6b** as an orange oil: NMR δ 7.31–6.94 (m, 7 H, ArH), 7.3 (d, 2 H, J = 8.7 Hz, meta signals), 6.83 (d, 2 H, J = 8.7 Hz, ortho signals), 6.65 (d, 2 H, J = 8.7 Hz, ortho signals), 5.75 (br s, 1 H, OH), 3.7 (s, 3 H, OCH_3), 3.5 (s, 1 H, cyclopropyl H). An analytical sample was obtained by drying over P_2O_5 at 60 °C for 6 h.

(*Z*)-1,1-Dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)-3-phenylcyclopropane (6c) was prepared in the same manner from **5d** (2 g, 4.2 mmol): NMR δ 7.55–6.83 (m, 8 H, ArH), 7.3 (s, 5 H, C_6H_5), 5.53 (br s, 1 H, OH), 3.69 (s, 3 H, OCH_3), 3.5 (s, 1 H, cyclopropyl H).

(*E*)- and (*Z*)-1,1-Dichloro-2-(4-hydroxyphenyl)-2,3-bis(4-methoxyphenyl)cyclopropane (8). The product from **4d** (0.5 g, 0.99 mmol) after hydrogenolysis crystallized from Et_2O–petroleum ether to give a white solid that tenaciously held Et_2O, even after drying (P_2O_5, 0.1 mmHg, 40 °C, 18 h), in an Abderhalden apparatus: mp 105–106 °C (dec, with prior softening). Elemental analysis and NMR showed 1.8 molecules of Et_2O per molecule of 8. Several recrystallizations from C_6H_6–hexane yielded 3.9 g of the product as a white powder: mp 96–97 °C dec; NMR δ 7.45 (d, 2 H, meta signals), 7.3–6.64 (m, 11 H, ArH + OH), 3.81 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.49 (s, 1 H, cyclopropyl H); FAB-MS m/z (% base) 419 (M + H + 4, 1.1), 417 (M + H + 2,

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2.1), 415 (M + H, 3.1), 381 (M + 2 - HCl, 33.6), 379 (-HCl, 100), 343 (-2HCl, 34).

2-Chloro-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-6-methoxyindene (10). The solution from hydrogenolysis of benzyl ether **9a** (0.75 g, 1.6 mmol) was filtered, concentrated, and purified on flash SiO_2 (gradient of 1:1 petroleum ether–CH_2Cl_2 to CH_2Cl_2) to yield an amber oil which was crystallized to give **10** as an orange-white powder: NMR δ 7.69 (d, 2 H, J = 8.7 Hz, meta signals), 7.3–6.69 (m, 9 H, ArH), 4.8 (s, 1 H, OH), 4.6 (s, 1 H, benzyl H), 3.87 (s, 3 H, OCH_3), 3.8 (s, 3 H, OCH_3).

Method D. (*Z*)-1,1-Dichloro-2,3-diphenyl-2-[4-[2-(dimethylamino)ethoxy]phenyl]cyclopropane, Dihydrogen Citrate Salt (7a). Phenol **6a** (1.25 g, 3.52 mmol), (dimethylamino)ethyl chloride hydrochloride (0.58 g, 4 mmol), and pulverized and flame-dried K_2CO_3 (1.38 g, 10 mmol) were suspended in 20 mL of dry Me_2CO under Ar and heated to reflux overnight. The resulting brown slurry was cooled to room temperature, filtered, and concentrated to give a brown oil. This was dissolved in 30 mL of Et_2O and washed with 1 N NaOH (2 × 40 mL). The combined aqueous layers were extracted with 25 mL of Et_2O. The combined organic layers were washed with 40 mL of brine and H_2O (2 × 20 mL), dried ($MgSO_4$), filtered, and concentrated to give a brown gum. The crude material was dissolved in MeOH–Et_2O, 2 mL of 30% HCl was added, and the mixture was stirred at room temperature for 0.5 h. The solvents were removed to give a brown glass. The glass was treated with 30% NaOH up to pH 11 and 50 mL of Et_2O was added. The organic layer was washed with 10% NaOH (2 × 50 mL), 50 mL of brine, and 50 mL of H_2O. The organic layer was dried (K_2CO_3), filtered, and concentrated to give a brown oil. This was loaded on a 20-mL basic Al_2O_3 (activity I) column and eluted with CH_2Cl_2. A yellow oil was collected which had spectral features of the desired product: NMR δ 7.73–7.02 (m, 12 H, ArH), 6.85 (d, J = 9 Hz, central ring 2 H ortho to O), 4.03 (t, J = 5.5 Hz, 2 H, OCH_2), 3.52 (s, 1 H, cyclopropyl H), 2.69 (t, J = 5.5 Hz, 2 H, CH_2N), 2.3 (s, 6 H, $N(CH_3)_2$). The oil was dissolved in hot EtOH and treated with excess anhydrous citric acid in hot EtOH. Treatment of the cooled solution with Et_2O yielded 0.11 g of **7a** in two crops as a white powder.

Method E. (*Z*)-1,1-Dichloro-2-[4-[2-(dimethylamino)ethoxy]phenyl]-2-(4-methoxyphenyl)-3-phenylcyclopropane (7b). Phenol **6b** (1.45 g, 3.78 mmol), (dimethylamino)ethyl chloride hydrochloride (1.63 g, 11.3 mmol), and dry, pulverized K_2CO_3 (5.2 g, 38 mmol) in 60 mL of dry Me_2CO were stirred and heated to reflux under Ar for 24 h. The resulting slurry was cooled to room temperature, filtered, and concentrated. The resulting oil was dissolved in 60 mL of EtOAc and washed with 10% NaOH (2 × 50 mL). The combined aqueous layers were extracted with 25 mL of EtOAc. The combined organic layers were washed with H_2O (2 × 50 mL), dried (K_2CO_3), filtered, and concentrated to give a dark oil, which was initially purified on 20 g of flash SiO_2 (9:1 C_6H_6–Et_3N), followed by two further purifications on flash SiO_2 (20 g each, Me_2CO) to give 225 mg of **7b** as a yellow gum: NMR δ 7.57–6.78 (m, 13 H, ArH), 4.08 (t, 2 H, J = 5 Hz, OCH_2), 3.8 (s, 3 H, OCH_3), 3.53 (s, 1 H, cyclopropyl H), 2.75 (t, 2 H, J = 5 Hz, CH_2N), 2.37 (s, 6 H, $N(CH_3)_2$); FAB-MS m/z (% base) 460 (M + 4 + H, 7), 458 (M + 2 + H, 18), 456 (M + H, 34), 422 (M + H - $H^{37}Cl$, 48), 420 (M + H - $H^{35}Cl$, 100). Further purification by flash SiO_2 (EtOAc) and drying over P_2O_5 yielded a sample suitable for combustion elemental analysis and high-resolution FAB-MS: $C_{26}H_{27}{}^{35}Cl_2NO_2$ + H calculated for 456.1497, found 456.1488.

Method F. 2-Chloro-3-[4-(benzyloxy)phenyl]-1-(4-methoxyphenyl)-6-methoxyindene (9a). Olefin **3d** (6 g, 14.2 mmol) and phenyl (bromodichloromethyl)mercury[21] (7 g, 15.9 mmol) were stirred at room temperature overnight in 75 mL of dry C_6H_6 under Ar. No reaction was evident (precipitation of PhHgBr) at this point, and the mixture was heated to reflux 1 h and then cooled to room temperature. Upon exposure to air, the resulting solution rapidly took on a deep blue color, and heat and HCl gas were evolved. The solution was cooled to room temperature, filtered, concentrated, and then dissolved in C_6H_{12} and refrigerated overnight to precipitate all mercury salts. The resulting brown solution was filtered through glass wool, concentrated, purified on 60 g of flash SiO_2 (9:1 petroleum ether–CH_2Cl_2), and crystallized to give indene **9a** as a white powder: NMR δ 7.6 (d, 2 H, J = 8.7 Hz, meta signals), 7.43 (s, 5 H, *O*-benzyl C_6H_5), 7.4–6.75 (m, 9 H, ArH), 5.15 (s, 2 H, OCH_2), 4.61 (s, 1 H, benzyl H), 3.8 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3); ^{13}C NMR δ 159.5, 159.0, 148.8, 138.0, 137.8, 135.0, 134.1, 130.5, 130.2, 129.8, 128.9, 127.8, 127.75, 126.2, 120.8, 115.1, 114.55, 112.5, 110.9, 70.3, 58.7, 55.7, 55.5.

Method G. 2-Chloro-1,3-bis(4-methoxyphenyl)-6-methoxyindene (9b). The crude trimethoxy cyclopropane **4e** (11 g, 23.3 mmol), from the phase-transfer dichlorocarbene addition reaction of olefin **3e** (10 g, 29 mmol) was dissolved in CH_2Cl_2, loaded on a 60-g SiO_2 flash chromatography column wetted with 1:1 petroleum ether–CH_2Cl_2, and eluted with a stepwise gradient of petroleum ether–CH_2Cl_2. A green band formed midway down the column and the first six 100-mL fractions contained a green eluate. The remaining fractions contained no desired cyclopropane as determined by TLC (SiO_2, 4:1 petroleum ether–CH_2Cl_2). Fractions 1–6 were combined and concentrated under reduced pressure. Upon breaking of the vacuum, the resulting green oil crystallized to give 8.42 g of **9b** as fine white needles which were recrystallized: NMR δ 7.67–6.73 (m, 11 H, ArH), 4.6 (s, 1 H, benzylic H), 3.89 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3); MS m/z (% base) 394 (M + 2, 7.7), 392 (M^+, 20.64), 357 (-Cl, 100).

Biological Assays. TAM was a gift of Stuart Pharmaceutical, Division of ICI Americas, Inc., Wilmington, DE. MER 25 was a gift of the Merrell Dow Research Institute, Division of Merrell Dow Pharmaceuticals, Inc., Cincinnati, OH. Absolute ethanol was obtained from U.S. Industrial Chemicals Co. Hormones and biochemicals were purchased from Sigma Chemical Co.

Animals and Housing. Viral-free immature female Swiss-Webster mice were obtained at 17–19 days of age from Sasco, weighing 8–10 g, and were used in the uterotrophic and antiuterotrophic assays. Immature female Sprague-Dawley rats, obtained at 17–19 days of age from Sasco, weighing 28–33 g, were used as sources of uteri for the estradiol receptor binding assay. Animals were housed in wire topped polycarbonate cages at six animals per cage, with the environment controlled at 25 °C and a 12 h light–dark cycle. The animals received a diet of Wayne Lab Blox rodent chow and tap water ad libitum.

Receptor-Binding Assay. The RBAs of the test compounds for the ER were determined by displacement of [^{3}H]estradiol from rat uterine cytosol in vitro. Female Sprague-Dawley rats (17–19 days old) were treated with 0.53 μg of estradiol in 0.1 mL of sesame oil for 3 consecutive days (total dose 1.6 μg). On the fourth day the rats were anesthetized with Et_2O and euthanized by cervical dislocation. A modification of Korenman's receptor binding assay method[47] was used. Single parallel incubations at each concentration of test compound and estradiol contained 4×10^{-6} diethylstilbestrol to distinguish between specific receptor binding and nonspecific protein–receptor binding of the compounds. Relative binding affinity of each compound was determined by the method of Bliss.[48]

Uterotrophic Assay. Estrogenic activity of the compounds was determined by using a modification[49] of the method of Rubin[50] using immature (17–19 days old) female Swiss-Webster mice. The test compounds were dissolved separately in a minimum amount of isopropyl myristate (IPM) and diluted serially with sesame oil to the proper concentrations (final concentration of IPM < 5%). Solutions were shaken at 25 °C for several hours to ensure complete dissolution. The mice were randomly separated into groups of six animals and weighed, and the compounds were administered by sc injection of 0.1 mL of the oil solutions into the nape of the neck for 3 consecutive days. The solutions were periodically checked by TLC to insure homogeneity. A control group received 0.1 mL of sesame oil alone. The animals were anesthetized with Et_2O and euthanized by cervical dislocation 24 h after the last injection. Body weights were determined and the uteri were removed, cleaned of adhering connective tissue and fat, blotted to remove tissue fluid, and weighed to the nearest 0.1 mg.

Antiuterotrophic Assay. Antiestrogenic activity of the compounds was determined by inhibition of estradiol-induced uterine weight gain in immature female Swiss-Webster mice.

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Animals were randomly distributed into groups of six animals. A modification[51] of the uterotrophic assay described above was used. Estradiol was dissolved in sesame oil (0.1 μg/mL). The test compounds were dissolved in IPM and diluted with IPM to achieve desired concentrations. The solutions were periodically checked by TLC to insure homogeneity. Injections were made in the nape of the neck for 3 consecutive days. The unstimulated control group received vehicles alone (0.05 mL of IPM and 0.1 mL of sesame oil each day), while the stimulated control group received 0.1 mL of the estradiol solution (total dose 0.03 μg). All test groups received 0.1 mL of the stimulating dose of estradiol (0.01 μg) plus 0.05 mL of the test compound solutions each day. The IPM and oil injections were made at separate sites to minimize possible physical or chemical interactions or reduced absorption of either compound. Antiestrogenic activity was measured as a decrease from the estradiol-induced increase in uterine weight seen in the test compound groups versus the estradiol-stimulated group alone.

Cell Culture. MCF-7 human breast cancer cells (obtained from the Michigan Cancer Foundation, Detroit) were grown as monolayer cultures at 37 °C in T-75 flasks in RPMI 1640 medium (without phenol red) supplemented with 2 mM L-glutamine, gentamicin (50 μg/mL), penicillin (100 units/mL), streptomycin (100 μg/mL), and calf serum (5%). Cultures were grown in a humid 5% CO_2 atmosphere and fed on alternate days. Exponential growth was maintained by subculturing at intervals when a level of (10–12) × 10^6 cells/T-75 flask was reached (8 days). Cells were trypsinized and plated in multiwell plates at a density of 7.5 × 10^4 cells/well in 3 mL of medium. The cells were allowed to attach for 2 days in the growth medium. The test compounds were dissolved in 11:9 PEG 400–EtOH and added to the culture medium on alternate days. The final concentration of vehicle was 0.1%. Control samples received vehicle alone at the same concentration. Cell growth was measured on day 4 by hemocytometric trypan blue exclusion. Percent inhibition was calculated as the ratio of the control mean cell count per well minus the treatment group mean cell count per well to the control mean cell count per well, multiplied by 100.

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Synthesis and Biological Activity of D_3-Trishomocubyl-4-amines

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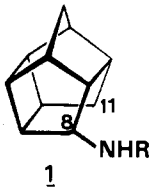
The D_3-trishomocubyl system was prepared from tertiary pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecan-8-ols **5** in one step by using a modified Ritter reaction yielding only one of the possible two geometrical isomers of 4-amino-3-alkyl (or aryl)-D_3-trishomocubane (**8**). Promising antagonism of reserpine-induced catalepsy was exhibited by these compounds which compared favorable with that of amantadine. Weak to mild anticholinergic properties were observed during the reduction of oxotremorine induced tremor and salivation procedure. Acute toxicities similar to that of amantadine were observed for some of these compounds. D_3-Trishomocubyl-4-amines appeared as a promising new class of anti-Parkinson agents.

Introduction

The tricyclic amantadine (1-aminoadamantane) is well-known for its clinical applications since the discoveries of its antiviral[1] and anti-Parkinson[2] properties. Numerous studies on the chemistry of various novel polycylic hydrocarbons have been conducted the past half-century.[3-6] However, research on the biological activities of these compounds have to a large extend been neglected. We recently reported the synthesis and pharmacological properties of a series of novel pentacyclic amines, namely pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecyl-8-amines (**1**).[7]



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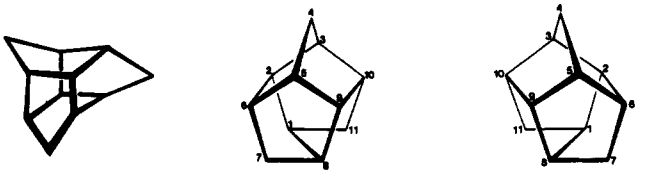
Promising anticataleptic activities were observed for some members in the series, while only weak to mild anticholinergic activities were noted. It was concluded that potential anti-parkinson properties of these pentacycloundecylamines were attributed by their effects on the dopaminergic system.[7] D_3-Trishomocubane (**2**), i.e. pentacyclo[6.3.0.^{2,6}.0^{3,10}.0^{5,9}]undecane, the most thermodynamically stable member[8] of all the possible pentacycloundecanes, has drawn the attention of many researchers because of its unique D_3 stereochemistry. Various authors[9-19] have reported the synthesis of D_3-trishomocubane and its derivatives during the past 20 years. This unique carbon skeleton, having chiral point group D_3 symmetry, intrinsic gyrochirality, and the stereoisomerism of D_3-trishomocubane have significant stereochemical implications, resulting in 3*R* and 3*S* stereoisomers for the racemic



2 **2 (3S)** **2 (3R)**

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