Synthesis and Receptor-Binding Affinity of Fluorotamoxifen, a Possible Estrogen-Receptor Imaging Agent

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Aminotamoxifen was totally synthesized from *p*-nitrobenzoyl chloride via a Friedel–Crafts acylation. Then, by means of a Balz–Schiemann reaction, aminotamoxifen was converted into fluorotamoxifen. The triazene variation of this conversion, with a 25% yield, enables a rapid, one-step diazotization, incorporating a fluorine atom into the phenyl ring of the tamoxifen. This reaction may be useful for the preparation of low specific activity ^{18}F-labeled tamoxifen, for distribution, and for estrogen-receptor studies. For these in vivo and in vitro studies, fluorotamoxifen was also synthesized from *p*-fluorobenzoyl chloride, and its chemical intermediates were compared with estradiol and hexestrol, for their receptor binding and competition, as well as for their uterotropic activity. It is demonstrated that tamoxifen and fluorotamoxifen are strong estradiol agonists and partial hexestrol agonists, while aminotamoxifen is a weak estradiol and hexestrol agonist.

Tamoxifen is a powerful nonsteroidal estradiol antagonist, useful for treating hormone-responsive human metastatic breast cancer,[1] as well as uterine, ovarian, and prostatic neoplasms.[2,3] It exerts both estrogenic and antiestrogenic effects through competitive binding to estrogen receptors, as demonstrated in most of the mammalian species studied so far.[4-7] γ-Labeling of steroidal and nonsteroidal moieties of high affinity to estradiol receptors is a goal in diagnostic nuclear medicine, aiming at a labeled product that will not hinder sterically the binding of the parent molecule to its receptors in the cancerous tissue. In this respect tamoxifen is a choice of clinical usefulness, and a fluorine atom is a most suitable radionuclide for its γ-labeling.

This work was undertaken in order to suggest a synthetic way to prepare labeled or unlabeled fluorotamoxifen in one step from its stable precursor aminotamoxifen. When labeled with ^{18}F, the γ-labeled [^{18}F]fluorotamoxifen is expected to be useful for following steroid-dependent tumor-ablation surgery and predicting response to antiestrogen therapy of such tumors, in spite of the fact that its binding sites are not restricted to estrogen target tissues.[8,9]

As isosteric exchange of hydrogen by fluorine usually elevates hydrophobicity and retards metabolism, ^{18}F-labeled compounds are considered near-physiologic probes for positron-emission tomography.[10] We report here synthesis, receptor binding capacity, and uterotropic activity of fluorotamoxifen[11] and of its precursor for ^{18}F incorporation by the Balz–Schiemann reaction : aminotamoxifen.

Results

Chemical Syntheses. The synthetic approach to obtain fluorotamoxifen and aminotamoxifen is shown in Schemes I and II. Friedel–Crafts acylation of *p*-fluorobenzoyl chloride gave 4-fluoro-4′-methoxybenzophenone (**1**) in 75% yield. Grignard reaction and dehydration of the tertiary alcohol formed gave the 1,1,2-triphenylbut-1-ene derivative (**2**) in 7% yield. The yield was much improved (30%) by reaction of **1** and propiophenone with $TiCl_4$–Zn reagent[12] and even better (55%) with McMurry $TiCl_3$–$LiAlH_4$ reagent.[13]

2 was smoothly demethylated with BBr_3 to the phenol **3** (92% yield), and finally the introduction of the basic side chain gave 1-(4-fluorophenyl)-1-[4-[2-(*N*,*N*-dimethylamino)ethoxy]phenyl]-2-phenyl-1-butene, known as fluorotamoxifen (**4**, Scheme I).

Isolation of the pure isomers **2*E*** and **2*Z*** was achieved by column chromatography and fractional crystallization (see below for X-ray and NMR assignments of configuration). However, each isomer gave on ether cleavage with BBr_3 mixture of **3*E*** and **3*Z***.[14] We could not separate the pure isomers of **3** and **4**. The synthesis of aminotamoxifen (**11**) was achieved in similar route from *p*-nitrobenzoyl chloride (Scheme II).

The conversion of aminotamoxifen (**11**) into fluorotamoxifen (**4**) was achieved by Balz–Schiemann reaction.[15] The classic method of thermal decomposition of aryldiazonium tetrafluoroborate salts seemed too drastic and tedious, and we chose the triazene variation which is milder and furthermore has the potential of giving higher radiochemical yields (Scheme III).

Extensive study of the triazene reaction and its application to fluorinated estrogens was published recently.[16]

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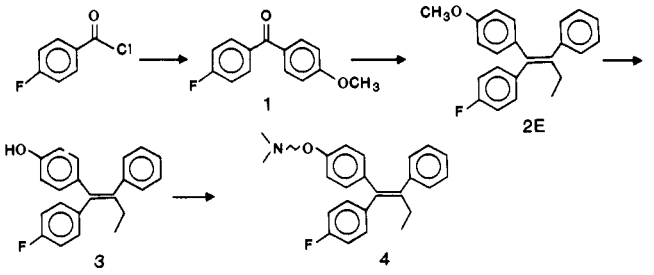
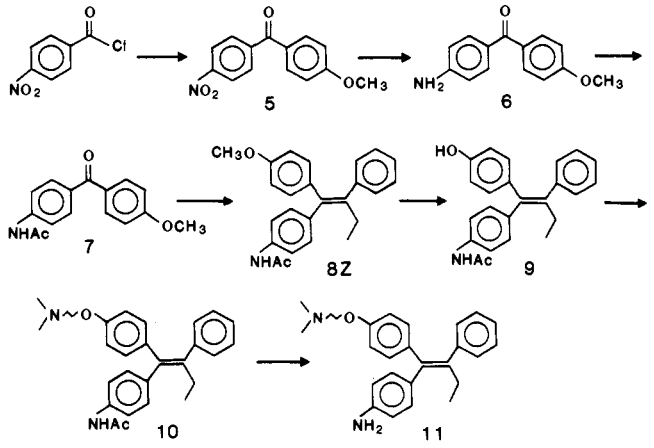
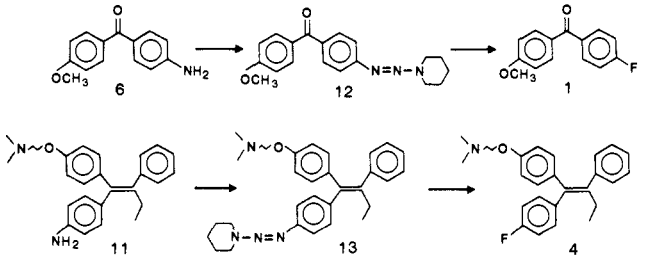
Scheme I



Scheme II



Scheme III



The yields of these reactions were found to be low to moderate. The ketone **6**, chosen as a model, was converted to the triazene **12** and to the fluoroketone **1** in 51% and 37% yields, respectively. Fluorotamoxifen (**4**) was obtained in 35% yield from the triazene **13** and 40% HF. This yield and the convenience of the procedure make the incorporation of fluorine into tamoxifen feasible and much more chemically efficient than by the Schiemann reaction.[16]

Analysis of Configuration and Conformation of the Triarylethylenes. NMR. In (*Z*)-tamoxifen, the resonances of all protons in the basic chain and ethyl group are at higher field compared to (*E*)-tamoxifen.[17] This relation holds for all tamoxifen derivatives and related triphenylethylenes prepared so far.[14,18-22] Always, substituents situated trans to a phenyl ring are shifted to lower fields (see Table I). On the basis of this rule, the resonances appearing at lower fields were assigned to the *E* isomers in compounds **8–11** and **13** (chemical shift difference is 0.15 ppm for OCH_2 and OCH_3, 0.10–0.15 ppm for CH_2N, and 0.06 ppm for $N(CH_3)_2$), while the aminoacetyl singlet in **8–10** *Z* isomers (which is para to the unsubstituted phenyl ring) appears at lower fields (Δ = 0.10 ppm). In compounds **2–4** the same pattern is followed, but here the *Z* isomer resonances are deshielded. The assignment was confirmed by X-ray study of **2*E***.

Table I. NMR Spectra of Tamoxifen Derivatives[a]

compd	OCH_2	NCH_2	$N(CH_3)_2$	CH_2	CH_3	NHAc
			Tamoxifen			
E[b]	4.02	2.68	2.28			
Z[b]	3.83	2.54	2.20			
E[c]	4.11	2.79	2.38	2.51	0.93	
Z[c]	3.94	2.64	2.28	2.47	0.92	
			Hydroxytamoxifen[d]			
E[e]	4.10	2.81	2.40		0.93	
Z[e]	3.95	2.70	2.33		0.93	
			Hydroxytamoxifen[f]			
E[g]	4.08	2.75	2.33			
Z[g]	3.93	2.64	2.27			
			Fluorotamoxifen			
Z[g]	4.09	2.75	2.35			
Z[h]	4.10	2.78	2.37	2.47	0.90	
E[g]	3.93	2.65	2.28			
E[h]	3.94	2.68	2.31	2.46	0.88	
10*E*	4.08	2.75	2.35	2.48	0.93	2.08
10*Z*	3.92	2.64	2.28	2.47	0.92	2.18
11*E*	4.10	2.77	2.37	2.47	0.90	
11*Z*	3.94	2.67	2.31	2.46	0.89	
13*E*	4.11	2.76	2.38	2.45	0.90	
13*Z*	3.96	2.63	2.34	2.44	0.89	

[a] In $CDCl_3$, values in ppm downfield from Me_4Si. [b] Reference 12. [c] Reference 13. [d] Phenyl ring on C_2 *p*-OH substituted. [e] Reference 14. [f] Phenyl ring on C_1 *p*-OH substituted. [g] Reference 6. [h] This work.

Table II. UV Spectra

compd	λ_{max},[a] nm (ϵ)
2*E*	241 (20 170), 281 (13 170)
2*Z*	240 (17 000), 280 (13 900)
3[b]	240 (17 400), 283 (12 150)
4[b]	240 (14 150), 280 (10 800)
14*E*[c]	243 (16 260), 282 (13 450)
14*Z*[c]	234 (11 130), 283 (9150)
cis-stilbene[d]	276 (11 700)
4-methoxy-*cis*-stilbene[d]	280 (10 600)
4-fluoro-*cis*-stilbene[d]	278 (10 200)
trans-stilbene[d]	296 (28 100), 305 (26 700)
4-methoxy-*trans*-stilbene[d]	306 (29 000), 310 (27 500)
1,1,2-triphenyl-1-butene[e]	223 (25 000), 268 (20 000)
tamoxifen[f]	238 (16 200), 277 (10 650), 288 (s, 10 400)
8[g]	258 (18 400), 285 (s, 13 000)
9[b]	257 (15 900), 288 (s, 10 800)
10[b]	257 (16 500), 285 (s, 11 600)
11[b]	228 (17 900), 253 (16 800), 294 (11 400), 340 (s, 5200)
13[b]	254 (15 400), 310 (s, 14 750), 352 (16 400)

[a] 95% EtOH. [b] 1:1 *E*:*Z* ratio. [c] Reference 16. [d] Reference 17. [e] Reference 9. [f] Commercial sample, Sigma, 1:1 *E*:*Z* ratio. [g] 2:1 *E*:*Z* ratio.

UV. UV spectra are not useful in assigning geometrical conformation for triphenylethylene isomers. Pure **2*E*** and **2*Z*** show only a minor difference in λ_{max} and ϵ values (see Table II). In 1-(4-methoxyphenyl)-1-propene (**14**) too, the two isomers **14*E*** and **14*Z*** have similar UV spectra.[22]

2–4, **14**, *cis*-stilbene, 4-methoxy-*cis*-stilbene, and 4-fluoro-*cis*-stilbene[23] show high wavelength bands at

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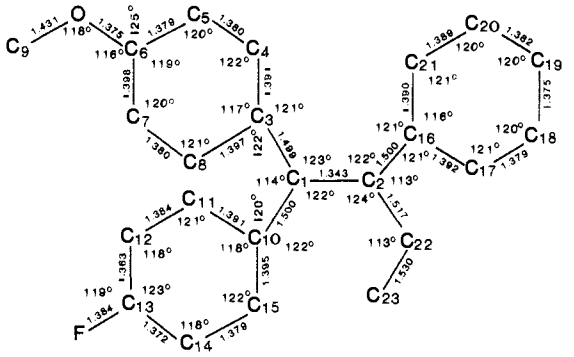

Figure 1. Atomic numbering, bond distances (Å), and bond angles (deg) in the fluorotamoxifen precursor **2*E***.

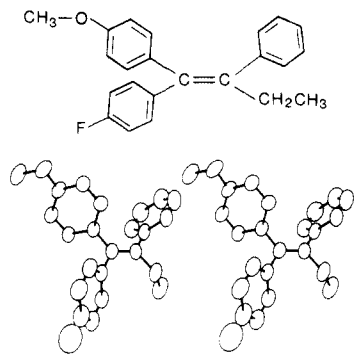

Figure 2. Computer-generated stereoscopic drawing of the X-ray-determined crystal structure of the fluorotamoxifen precursor **2*E***. Hydrogen atoms were omitted for clarity.

276–283 μm (ε 10 000–14 000), which is different from that of *trans*-stilbene or 4-methoxy-*trans*-stilbene: 296–310 μm (ε 27 000–29 000).[23] This seems to indicate that the high band is due to a "*cis*-stilbenic chromophore". However, the molecules are not planar but rather have propellar shape, and the "*cis*-stilbenic" assumption has led indeed to erroneous assignment in the clomiphene series.[24,25]

Tamoxifen shows bathochromic shift to 288 μm, but **8–10** have their high band at 285–288 μm too, i.e. with no apparent contribution from the *p*-NHAC chromophore, while **11** and **13** show strong bathochromic shifts at their high wavelength bands (Table II).

The conclusion is that the UV spectra of 1,1,2-triphenyl-1-butene derivatives is composed of contributions from the aryl rings, each rotated along its 1,4-axis, without stilbenic chromophore contributions.

X-ray. For absolute confirmation of the geometric conformation we studied the X-ray spectra of **2*E***. In accordance with the NMR spectrum, the unsubstituted phenyl ring and the *p*-fluorophenyl ring are trans to each other. The atomic numbering scheme and bond lengths and angles in the **2*E*** molecule are given in Figure 1.

2*E* has propellar-like configuration (see Figure 2), with angles between rings (Table III), bond lengths, and bond angles similar to those of tamoxifen and to related compounds (see ref 21 and 26 and references cited there).

Crystal Data: colorless crystal of **2*E***; $C_{23}H_{21}OF$; fw 332.4; grown by slow evaporation of a methanol solution; space group $P\bar{1}$; a = 10.107, b = 10.683, c = 9.682 Å; α = 114.00, β = 105.56, γ = 77.42°; V = 913 Å^3; Z = 2; ρ_{calcd} = 1.21 g cm^{-3}, μ(Mo Kα) = 0.45 cm^{-1}; collection range $3 \leq 2\theta \leq 48$. A total of 2839 unique reflections was measured, of which 2286 were considered to be observed, $I \geq 3\sigma(I)$, R = 0.051, R_w = 0.075.

Table III. Comparison of Stereochemical Features

feature	**2*E***	tamoxifen[b]	clomiphene[b]
O···C=C dist, Å	6.07	6.06	6.07
dihedral angles,[a] deg			
R_1-R_2	65	59	67
R_1-R_3	97	87	78
R_2-R_3	60	57	65

[a] Angles between ring normals. R_1 = phenyl (or *p*-fluorophenyl in **2*E***) ring on $C_1 \geq R_2$ = phenyl ring on $C_2 \geq R_3$ = basic side chain substituted (or *p*-methoxy in **2*E***) phenyl ring on C_1. [b] Reference 20.

Biological Activity and Discussion

The uterotropic assay for 0.02–10.0 μg/d of estradiol and 0.05–0.5 μg/d of hexestrol in 21-day-old immature rats demonstrated a dose-related increase in uterine wet weight. The striking feature of the "Sabra" rat strain used in our experiments is the significantly higher intrinsic activity of hexestrol in comparison to estradiol (Tables IV and V), and the saturation dose of estradiol in our rats differs remarkably from those of Low and Katzenellenbogen[27] and Jordan and Gosden.[28] For that reason 0.2 μg/d was chosen as the physiological saturation dose of estradiol and 0.1 μg/d as the saturation dose of hexestrol. The corresponding saturation doses of tamoxifen, aminotamoxifen, and fluorotamoxifen as well as of three intermediates are given in Table V. In this test tamoxifen and fluorotamoxifen were found to be highly agonistic with estradiol, and partial agonistic as compared to hexestrol, while aminotamoxifen was a partial weak agonist to both estradiol and hexestrol (Tables IV and V). Obviously, the agonistic/antagonistic capacity of tamoxifen, aminotamoxifen, and fluorotamoxifen depended on the saturation doses of estradiol and hexestrol chosen: the higher the saturation doses—the lower was the estrogenic activity of tamoxifen and fluorotamoxifen. The reason for the partial estrogenic response of these compounds is due either to differences associated with the transformation reaction of the cytoplasmic estrogen receptor[29] or to their lower capacity to induce a positive cooperative interaction with the estrogen receptors, as has been shown for estriol and estrone.[30,31]

Under the specific conditions of our experiments, two types of binding sites could be distinguished: type I has a dissociation constant (K_{DI}) of 1.2 nM and constitutes approximately 0.2–0.3 pm/uterus, while type II has a dissociation constant (K_{DII}) of 7.3–7.5 nM and constitutes 0.2–0.35 pm/uterus (Figure 3). Similar values were obtained by Clark et al.,[32] who also found two types of receptive sites with K_{DI} of 0.8 nM and K_{DII} of 30 nM and with a fourfold value in number of binding sites (4 pm/uterus vs. 1 pm/uterus in type I sites). The differences between the numerical values in the two reports are attributable to differences in animal strain and to some

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Table IV. Uterotropic Assay of Tamoxifen, Aminotamoxifen, and Fluorotamoxifen in "Sabra" Strain Rats, as Compared to That of Estradiol and Hexestrol

compd	dose, μg/d	uterine wet wt (gp mean ± SEM)
	Estradiol (E)	
estradiol	0.04	41.9 ± 1.9
estradiol	0.08	54.4 ± 1.8
estradiol	0.10	58.1 ± 3.0
estradiol	0.16	70.2 ± 2.8
estradiol	0.20	76.3 ± 2.4
estradiol	1.00	74.6 ± 2.6
estradiol	10.00	74.2 ± 4.3
	Hexestrol (H)	
hexestrol	0.01	29.3 ± 2.3
hexestrol	0.03	62.6 ± 2.1
hexestrol	0.1	86.8 ± 2.2
hexestrol	0.3	94.5 ± 3.3
hexestrol	1.0	90.2 ± 4.1
hexestrol	10.0	97.3 ± 4.5
	Tamoxifen (T)	
tamoxifen	0.1	31.4 ± 1.4
tamoxifen	1.0	50.7 ± 3.6
tamoxifen	10.0	65.0 ± 1.9
tamoxifen	50.0	66.0 ± 2.6
estradiol	0.10	53.0 ± 2.8
E 0.10 + T	50.0	64.7 ± 2.4
estradiol	0.16	65.8 ± 3.7
E 0.16 + T	1.0	62.6 ± 3.8
E 0.16 + T	10.0	65.5 ± 2.5
estradiol	0.20	69.2 ± 3.2
E 0.20 + T	50.0	64.2 ± 2.1
estradiol	10.0	68.8 ± 4.8
E 10.0 + T	50.0	70.7 ± 2.7
hexestrol (H)	0.05	50.2 ± 0.8
H 0.05 + T	50.0	65.0 ± 1.7
hexestrol (H)	0.5	93.0 ± 1.8
H 0.5 + T	50.0	66.0 ± 1.6
	Aminotamoxifen (A-T)	
A-T	1.0	29.6 ± 2.1
A-T	10.0	44.0 ± 1.7
A-T	100.0	50.3 ± 2.0
A-T	500.0	54.0 ± 2.1
estradiol	0.08	52.8 ± 2.7
E 0.08 + A-T	1.0	58.2 ± 2.5
E 0.08 + A-T	10.0	54.8 ± 0.4
E 0.08 + A-T	500.0	56.5 ± 2.2
estradiol	0.10	53.0 ± 2.8
E 0.10 + A-T	100.0	46.0 ± 1.3
estradiol	0.2	69.2 ± 3.2
E 0.2 + A-T	100.0	43.0 ± 0.9
E 0.2 + A-T	500.0	62.0 ± 1.4
estradiol	10.0	68.0 ± 2.1
E 10.0 + A-T	100.0	57.1 ± 2.0
E 10.0 + A-T	500.0	67.2 ± 3.4
hexestrol (H)	0.05	50.2 ± 0.8
H 0.05 + A-T	100.0	46.0 ± 2.1
hexestrol (H)	0.5	93.0 ± 1.8
H 0.5 + A-T	100.0	70.3 ± 1.9
	Fluorotamoxifen (F-T)	
fluorotamoxifen	10.0	68.9 ± 2.1
fluorotamoxifen	100.0	76.4 ± 1.7
fluorotamoxifen	1000.0	85.7 ± 4.3
estradiol	0.16	68.2 ± 2.8
E 0.16 + F-T	10.0	72.2 ± 2.7
E 0.16 + F-T	1000.0	76.9 ± 2.2
estradiol	0.1	53.0 ± 2.8
E 0.1 + F-T	100.0	74.0 ± 1.9
estradiol	0.2	77.1 ± 4.1
E 0.2 + F-T	100.0	73.8 ± 2.0
estradiol	10.0	75.5 ± 2.2
E 10.0 + F-T	100.0	80.3 ± 2.6
hexestrol (H)	0.05	56.1 ± 2.0
H 0.05 + F-T	100.0	70.4 ± 1.2
hexestrol	0.5	99.6 ± 1.8
H 0.5 + F-T	100.0	71.2 ± 3.5
solvent (saline)	0.2[a]	29.7 ± 2.2

[a] In mL.

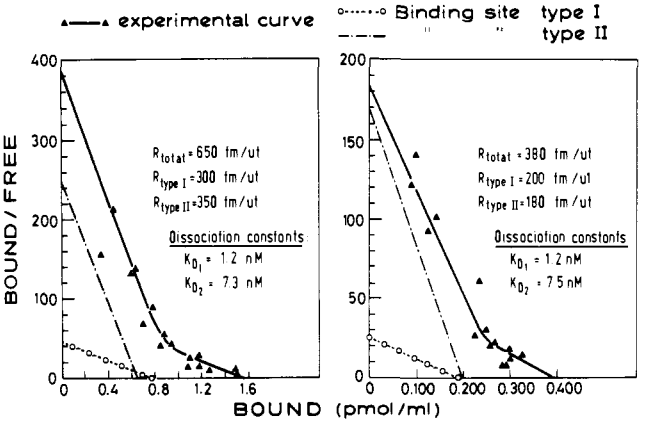

Figure 3. Saturation analysis of [^{3}H]estradiol binding to rat uterine cytosol. Range of [^{3}H]estradiol used: (left) 1–160 nM; (right) 1-40 nM. Data plotted according to Scatchard and corrected according to Rosenthal and Feldman.[42-44] R_{total} = receptor total.

Table V. Uterotropic Effect of Tamoxifen, Fluorotamoxifen, and Four of Its Chemical Intermediates, as Compared to That of Estradiol and Hexestrol[a]

compd	min satn dose, μg/d	max uterine wet wt, mg	percent estradiol effect
saline	0.2[b]	29.7 ± 2.2	39
estradiol	0.2	76.3 ± 2.4	100
hexestrol	0.1	62.6 ± 2.1	143
tamoxifen	10.0	65.0 ± 1.9	93
fluorotamoxifen (**4**)	100.0	76.4 ± 1.7	108
aminotamoxifen (**11**)	500.0	54.0 ± 2.1	76
3 (Scheme I)	10.0	97.0 ± 2.9	140
9 (Scheme II)	50.0	65.8 ± 2.8	98
10 (Scheme II)	50.0[c]	41.8 ± 2.3	60

[a] Chemical's designated numbers refer to Schemes I and II. [b] In mL. [c] The only dose tested.

Table VI. A_{50} Inhibition Values and Dissociation Constants (K_A) of Tamoxifen, Fluorotamoxifen, and Some of Its Chemical Intermediates as Compared to Those of Diethylstilbestral (DES)[a]

compd	A_{50}, M	K_A, M
DES	4×10^{-9}	0.4×10^{-9}
tamoxifen	2×10^{-6}	2.1×10^{-7}
fluorotamoxifen (4)	5×10^{-6}	5.3×10^{-7}
aminotamoxifen (11)	2×10^{-8}	1.9×10^{-9}
3 (Scheme I)	6.7×10^{-7}	6.3×10^{-8}
9 (Scheme II)	1.2×10^{-6}	1.1×10^{-7}

[a] See Figure 4 for graphic representation. Chemical's designated numbers refer to Schemes I and II.

secondary experimental conditions.

The A_{50} values (concentration of cold ligand that inhibits 50% of the [^{3}H]-E_2 specific binding) and K_A values (dissociation constants of the cold ligands from their complexes) are given in Table VI. From this table it can be seen that in the presence of 2×10^{-6} M tamoxifen there is a 50% inhibition of [^{3}H]-E_2 binding, while fluorotamoxifen demonstrates the same activity at 5×10^{-6} M. The displacements curves for the four test compounds are given in Figure 4.

An isomeric 2-[(4-aminophenyl)amino]tamoxifen was used recently[33,34] as a precursor for preparing its corresponding isomers of iodotamoxifen for internal imaging. The authors assumption that the iodine atom "would not

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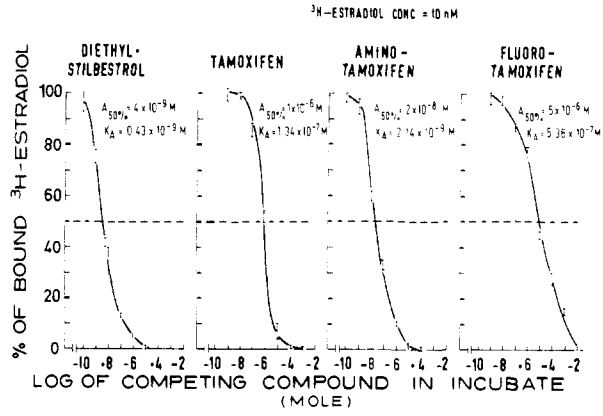

Figure 4. Competitive inhibition of [^{3}H]estradiol binding to rat uterine cytosol by diethylstilbestrol (DES), tamoxifen, aminotamoxifen, and fluorotamoxifen.

seriously alter the affinity of the molecule for estrogen receptors" is, in our opinion, erroneous. Also, Hanson and Seitz,[35] who studied the tissue distribution of [^{125}I]tamoxifen (with the radioiodine situated ortho to the basic side chain) concluded that "radioiodinated tamoxifen is a poor candidate as a potential radiodiagnostic effort for estrogen-responsive tissues".

A major advantage of using fluorotamoxifen, in addition to its steric resemblance to the parent compound, is its metabolic stability, as the fluorine atom we inserted in the tamoxifen molecule is in the same position where one of the hydroxylative metabolism occurs, thus preventing the tamoxifen metabolism through one specific route. Moreover, in a comparative study of the various ring-halogenated hexestrols it has been shown that fluorinated hexestrols had a higher binding affinity for uterine estrogen receptors than the other halogenated hexestrols, rating at a level of up to 205% as compared to 300% for hexestrol and 100% for estradiol.[36,37] These results stimulated us to carry the present study.

Experimental Section

Elemental analyses were done at The Hebrew University of Jerusalem Microanalyses Laboratory. Melting points were taken on a Fisher-Johns melting point apparatus and were presented uncorrected. UV spectra were determined with a Gilford 2400-S spectrophotometer. IR spectra were obtained with a Perkin-Elmer 157G spectrometer. ^{1}H and ^{19}F NMR spectra were recorded on a Bruker WH-300 pulsed FT spectrometer, in $CDCl_3$, unless otherwise indicated. Chemical shifts are reported in ppm downfield from the interval Me_4Si signal and upfield from the interval $CFCl_3$ signal for ^{1}H and ^{19}F, respectively. Electron impact mass spectra were recorded on a MAT 311 instrument.

Chromatography columns were packed with Merck 35-70 silica gel or dry silica (Woelm-Pharma) and eluted successively with hexane, hexane–CH_2Cl_2, CH_2Cl_2, and CH_2Cl_2–CH_3OH. Solvents obtained from Frutarom were used without purification. TLC was taken on Merck silica gel GF_{254} plates (0.25-mm thickness). *p*-Fluorobenzoyl chloride, *p*-nitrobenzoyl chloride, and pyridine hydrofluoride were purchased from Aldrich.

In this study, "workup" means diluting with H_2O, extracting with CH_2Cl_2, drying the organic phase with $MgSO_4$, filtering, and evaporating to dryness.

X-ray Crystal Structure Determination. Data were collected on a PW 1100/20 Philips four-circle computer-controlled diffractometer. Mo Kα (λ = 0.71069 Å) radiation with a graphite crystal monochromator in the incident beam was used. The unit cell dimensions were obtained by a least-squares fit of 20 centered reflections in the range of $10° < \theta < 13°$. Intensity data were measured by using the ω-20 technique. The scan width, $\Delta\omega$, for each reflection was 1° with a scan time of 20 s. Background measurements were made at both limits of each scan. Three standard reflections were monitored every 60 min. No systematic variations in intensities were noticed.

Intensities were corrected for Lorentz and polarization effects. All non-hydrogen atoms were found by using the results of the MULTAN direct-method analysis.[38] After several cycles of refinement,[39] the positions of the hydrogen atoms were calculated and introduced with a constant isotropic temperature factor of 0.5 Å^2. Refinement proceeded to convergence by minimizing the function $\sum w(|F_o| - |F_c|)^2$, where the weight w is $\sigma(F_o)^{-2}$. The discrepancy indices $R = \sum(|F_o| - |F_c|/\sum|F_o|$ and $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$ are presented with other crystal data as supplementary material for this paper.

4-Fluoro-4′-methoxybenzophenone (1). *p*-Fluorobenzoyl chloride (40 ml; 53.6 g, 0.33 mol) and 45 mL (0.34 mol) of anisole were dissolved in 150 mL of CS_2. Dry $AlCl_3$ (40 g) was added in portions while stirring vigorously at room temperature. Toward the end of the addition, a red complex precipitated. After 1 h at room temperature the reaction was decomposed with 100 mL of concentrated HCl and ice. Filtering and washing with water yielded a pink solid that was recrystallyzed from benzene–hexane to yield white crystals: 59 g (75%); mp ~96 °C; R_f (C_6H_6) 0.55; IR (KBr) 1640 cm^{-1}; λ_{max} (EtOH) 221 μm (9600), 289 (12550); ^{1}H NMR δ 3.87 (3 H, s, OCH_3), 7.85–6.99 (8 H, m, including AB spectra at 7.77 and 7.06 ppm with J_{Ab} = 9 Hz for the methoxy ring); ^{19}F NMR ($CDCl_3$) 107.05 (from $FCCl_3$, 7-line m). Anal. Calcd for $C_{14}H_{11}FO_2$: C, 73.04; H, 4.78; F, 8.26. Found: C, 73.24; H, 5.08; F, 7.62.

1-Bromo-1-phenylpropane. To 60 g (0.44 M) of 1-phenylpropanol in 200 mL of CH_2Cl_2, cooled in ice, were dropped 30 mL of PBr_3. At the end of the addition, the reaction was stirred overnight at room temperature and worked up. Distillation at 84 °C (4 mm) gave 51 g (58%) of white liquid. On standing, it turns brown, with HBr evolution. ^{1}H NMR ($CDCl_3$) δ 0.9 (3 H, t, J = 6 Hz), 2.1 (2 H, m), 4.73 (1 H, *t*, J = 7 Hz), 7.1 (5 H, narrow m).

1-(4-Fluorophenyl)-1-(4-methoxyphenyl)-2-phenyl-1-butene (2). A. Grignard Reagent. Grignard reagent was prepared from 29.9 g (150 mM) of 1-bromo-1-phenylpropane and 3.6 g (150 mM) of Mg in 400 mL of dry ether. Solid fluoro ketone 1 (11.4 g, 50 mM) was added, and after the slight reaction subsided, it was refluxed for 4 h. Decomposing on ice and dilute HCl and workup gave 10 g of oily solid. Acetic acid (50 mL) and 10 mL of concentrated H_2SO_4 were added, and the dark solution was stirred for 3 h at room temperature and worked up. Chromatography on silica gel gave 1.2 g (7% yield) of a white solid, a mixture of ***2E*** and ***2Z***.

A second chromatography on dry silica gel, 60 × 2 cm, and elution with hexane–20% CH_2Cl_2 in hexane gave separation of the geometrical isomers, pure after recrystallization from hexane, 0.3 g each. ***2E***, the more polar isomer: R_f (1:4 CH_2Cl_2–hexane) 0.45; mp 125 °C; IR ($CHCl_3$) 1610 cm^{-1}; UV λ_{max} (EtOH) 241 μm (20170), 281 (13170); ^{1}H NMR δ 0.92 (3 H, t, J = 7.4 Hz), 2.45 (2 H, q, J = 7.4 Hz), 3.66 (3 H, s, OCH_3), 6.55, 6.76 (4 H, AB q, J_{AB} = 8.5 Hz, OCH_3 ring protons), 6.78–7.22 (9 H, m). Anal. Calcd for $C_{23}H_{21}FO$: C, 83.13; H, 6.32; F, 5.70. Found: C, 83.01; H, 6.22; F, 5.81. Mass spectrum m/e 332 (M^+, 100%), 317 ($M - CH_3$, 51%), 303 ($M - C_2H_5$, 8%), 301 ($M - OCH_3$, 7%), 272 ($M - C_2H_5 - OCH_3$, 4%) 255 ($M - C_6H_5$, 10%), 240 ($M - Ph - CH_3$, 20%), 222 (10%), 210 (35). ***2Z***, the less polar isomer: R_f 0.50; mp 104 °C; IR ($CHCl_3$)

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1610 cm^{-1}; UV λ_{max} (EtOH) 228 μm (s, 16700), 240 (17000), 280 (13900); ^1H NMR δ 0.94 (3 H, t, J = 7.4 Hz, CH_2CH_3), 2.49 (2 H, q, CH_2CH_3, J 7.4 Hz), 3.81 (3 H, s, OCH_3), 7.20–6.67 (13 H, m); identical with E isomer with slight variations of relative intensity.

B. $TiCl_3$. To 25 g of $TiCl_3$–$LiAlH_4$ (McMurry reagent) in 250 mL of dry THF stirred under N_2 was added 3.9 g (17 mM) of fluoro ketone 1 and 3 g (22 mM) of propiophenone in 100 mL of THF. After the slight exothermic reaction subsided, it was refluxed for 4 h, decomposed with ice, and extracted with benzene. Workup gave 5.6 g of oily solid that consists of 62:38 E:Z ratio with small amounts of impurities (propiophenone dimer and ketone dimer). Recrystallization from hexane gave 0.9 g of pure E isomer and 2.2 g of a mixture of E and Z; yield 3.1 g (55%).

C. $TiCl_4$–Zn. To the reagent prepared from 33 g (175 mM) of $TiCl_4$ and 23 g (350 mM) of Zn powder, in 400 mL dry ether under N_2, were added 12 g (51 mM) of 1 and 9 g (66 mM) of propiophenone in 100 mL of ether. After 16-h stirring at room temperature and 4-h reflux, it was worked up. Chromatography gave 5.2 g (30% yield) of white solid, 3:1 **2*E*:2*Z*** ratio by NMR.

1-(4-Fluorophenyl)-1-(4-hydroxyphenyl)-2-phenyl-1-butene (3). 2*E* (0.8 g, 2.4 mM) in 30 mL of CH_2Cl_2 and 2 mL of BBr_3 (21 mM) were stirred for 1 h at room temperature, decomposed with ice, and worked up. The oil was recrystallized from hexane to give 0.65 g of white solid: mp 123 °C; yield 85%; R_f (7:3 CH_2Cl_2–hexane) 0.2, R_f (CH_2Cl_2) 0.6. NMR shows 55:45 E:Z isomer ratio, and therefore BBr_3 caused E to Z isomerization. IR (neat) 3400, 3000, 1610 cm^{-1}; UV λ_{max} (EtOH) 240 μm (17400), 283 (12150); ^1H NMR δ 0.93, 0.92 (2 overlapping t, J = 7.3 Hz, 3 H, 55:45), 2.48, 2.46 (2 overlapping q, J = 7.3 Hz, 2 H), 6.46–7.23 (m, 13 H). Anal. Calcd for $C_{22}H_{19}FO$: C, 83.01; H, 5.97; F, 5.97. Found: C, 83.12; H, 6.03; F, 5.85. Mass spectrum m/e 318 (M^+, 100%), 303 (M – CH_3, 33%), 289 (M – C_2H_5, 6%), 241 (M – C_6H_5, 8%), 226 (M – CH_3 – C_6H_5, 20%), 210 (23%), 218 (10%), 202 (19%), 184 (9%), 107 ($C_6H_5OCH_3^+$, 10%), 91 (tropylium, 9%). Reaction of a 6:4 **2*E*:2*Z*** ratio (7.7 g, 23 mM) with BBr_3 (5 mL, 50 mM) gave 7 g (95% yield) of a 1:1 **3*E*:3*Z*** ratio.

1-(4-Fluorophenyl)-1-[4-[2-(*N*,*N*-dimethylamino)ethoxy]phenyl]-2-phenyl-1-butene (4). 3 (1:1 E:Z ratio; 7 g, 22 mM) and 3.4 g (60 mM) of KOH were dissolved in 350 mL of ethanol. After 10 min, 5.8 g (40 mM) of 2-(dimethylamino)ethyl chloride hydrochloride were added and the reaction was stirred and refluxed for 20 h. Workup and chromatography on silica gel gave 2 g of recovered **3** and 2.2 g of waxy solid; 28% (40%) yield; R_f (8% CH_3OH in CH_2Cl_2) 0.2 (SM = 0.9); IR ($CHCl_3$) 3450, 2960, 1600, 1500 cm^{-1}; UV λ_{max} (EtOH) 240 μm (14150), 280 (10800); ^1H NMR δ 0.88 (E), 0.90 (Z, 3 H, 2 overlapping t, J = 7.2 Hz), 2.37 (Z), 2.31 (E, 6 H, s, N,N-dimethyl), 2.47 (Z), 2.46 (E, 2 H, 2 overlapping q, J = 7.2 Hz, CH_2), 2.78 (Z), 2.68 (E, 2 H, 2 overlapping t, J = 5.9 Hz, CH_2N) 4.10 (Z), 3.94 (E, 2 H, 2 overlapping t, J = 5.9 Hz, CH_2O), 7.25–6.54 (13 H, m aromatic). Anal. Calcd for $C_{26}H_{28}FNO$: C, 80.20; H, 7.20; N, 3.60; F, 4.88. Found: C, 83.24, H, 7.52, N, 3.00. mass spectrum m/e 389 (M^+, 4%), 356 (3%), 344 (5%), 342 (12%), 149 (8%) 72 (>N^+—C=, 35%), 58 (>N^+=, 100%).

4-Nitro-4′-methoxybenzophenone (5). To 40 g (0.22 mol) of p-nitrobenzoyl chloride and 25 g (0.22 mol) of anisole in 150 mL of CS_2 was added in portions 25 g of dry $AlCl_3$. A strong reaction ensued, and a red complex precipitated. After 15 min the whole reaction solidified into a cake. After standing for 1 h at room temperature, the complex was decomposed with 80 mL of concentrated HCl and ice water. The pink solid was filtered and washed thoroughly with water. Recrystallization from benzene–hexane yielded 40 g (72% yield) of a white solid: mp 123 °C (lit. mp 121 °C); R_f (C_6H_6) 0.5, R_f (CH_2Cl_2) 0.9. The reaction was run on a scale of up to 100 g, and the average yield was 70–80%. IR (KBr) 1640 cm^{-1}; UV λ_{max} (EtOH) 266 μm (14750), 298 (10950); ^1H NMR δ 3.90 (3 H, s, OCH_3), 6.99, 7.81 (4 H, AB q, J_{AB} = 9 Hz, aromatic OCH_3 ring), 7.89, 8.34 (4 H, AB q, J_{AB} = 9 Hz, aromatic NO_2 ring).

4-Amino-4′-methoxybenzophenone (6). Ketone **6** (10 g, 39 mmole) and $Na_2S_2O_4 \cdot H_2O$ (30 g, 156 mmol) in 250 mL of EtOH were refluxed overnight. Water was added and the reaction worked up. Recrystallization from benzene–hexane gave an oily solid, 5.5 g (62% yield), that solidified upon standing: mp ~110 °C; R_f (CH_2Cl_2) 0.2. It turns pink on standing. The amine is quite soluble in water, and with larger batches it was noticed that continuous liquid–liquid extraction with EtAc raised the yield. IR ($CHCl_3$) 3420, 3000, 1640, 1620 cm^{-1}; UV λ_{max} (EtOH) 223 μm (10900), 248 (9800), 279 (12000) 329 (18350); ^1H NMR δ 3.86 (3 H, s, OCH_3), 4.3 (2 H, m, NH_2), 6.60, 7.63 (4 H, AB q, J_{AB} = 9 Hz, aromatic amino ring protons), 6.90, 7.70 (4 H, AB q, J_{AB} = 7.0 Hz, aromatic methoxy ring protons). Anal. Calcd for $C_{14}H_{13}NO_2$: C, 74.00; H, 5.73; N, 6.16. Found: C, 74.18; H, 5.79; N, 6.08.

4-(Acetylamino)-4′-methoxybenzophenone (7). Amine **6** (5.5 g) was dissolved in 45 mL of Ac_2O and 5 mL of pyridine and heated to 110 °C for 5 min. After standing overnight and decomposing with water, a white solid precipitated. It was filtered and washed thoroughly with water. Recrystallization from benzene gave 3 g of white, wooly needles: mp 172 °C; 60% yield; R_f (9:1 CH_2Cl_2–CH_3OH) 0.5. Larger batches with continuous extraction of the amine with ethyl acetate and direct acelytation gave a combined yield of 30% from the nitro ketone **5**: IR (KBr) 3340, 3630–2960, 1660, 1640 cm^{-1}; UV λ_{max} (EtOH) 229 μm (13200), 301 (25600); ^1H NMR δ 2.22 (3 H, s, amino acetyl), 3.89 (3 H, s, OCH_3), 6.96, 7.66 (4 H, AB q, J_{AB} = 9.0 Hz, aromatic methoxy ring), 7.73, 7.80 (4 H, AB q, J_{AB} = 9.0 Hz, aromatic aminoacetyl ring), 8.33 (m, 1 H, NH_2). Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.37; H, 5.57; N, 5.50. Found: C, 71.44; H, 5.64, N, 5.16. Mass spectrum m/e 269 (M^+, 100%), 227 (M – ketene, 78%), 196 (M – ketene – OCH_3, 15%), 135 (M – C_6H_5 – NHAc, 56%), 120 (135 – CH_3, 56%), 92 (120 – C=O, 31%).

1-[4-(Acetylamino)phenyl]-1-(4-methoxyphenyl)-2-phenyl-1-butene (8). To 20 g of $TiCl_3$–$LiAlH_4$ (McMurry reagent) in 150 mL of dry THF, under N_2, was added dropwise a solution of 4.5 g (16.7 mmol) of ketone **7** and 3 g (22 mmol) of propiophenone in 50 mL of THF. A slight exothermic reaction occurred. The black suspension obtained was stirred for 20 h at room temperature and decomposed with ice. Extraction with ether–benzene and the usual workup gave a yellow oil that was chromatographed on silica gel. white solid (2.5 g) was obtained: mp 105–110 °C; yield 40%. The solid is a 2:1 E:Z ratio according to NMR: IR ($CHCl_3$) 2960, 1690, 1610 cm^{-1}; UV λ_{max} (EtOH) 258 μm (18400), 285 (s, 13000); ^1H NMR δ 0.92, 0.93 (3 H, 2 overlapping t, J = 7.3 Hz, CH_2CH_3), 2.09, 2.19 (3 H, s, 2:1 E:Z ratio, aminoacetyl protons), 2.48, 2.50 (2 H, 2 overlapping q, J = 7.3 Hz, CH_2CH_3), 3.68, 3.83 (3 H, s, 1:2 Z:E ratio, OCH_3), 6.53–7.25 (m, 13 H). Anal. Calcd for $C_{25}H_{25}NO_2$: C, 80.86; H, 6.74; N, 3.77. Found: C, 80.62; H, 6.70; N, 3.82. Mass spectrum m/e 371 (M^+, 100%), 356 (M – CH_3, 71%), 342 (M – Et, 11%), 341 (12%), 340 (M – OCH_3, 13%), 329 (M – CH_2=C=O, 7%), 328 (M – Ac, 8%), 314 (M – CH_2CO – CH_3, 26%), 254 (26%), 248 (19%), 222 (38%).

1-[4-(Acetylamino)phenyl]-1-(4-hydroxyphenyl)-2-phenyl-1-butene (9). 8 (2.5 g, 6.7 mM) was dissolved in 100 mL of CH_2Cl_2 and 2.5 mL of BBr_3 added. The black solution was stirred for 1.5 h at room temperature and worked up as usual to give white solid, 1.3 g (54%). BBr_3 causes isomerization. A 2:1 E:Z ratio in the starting material yields a 1:1 E:Z ratio in the product. The phenol is somewhat sensitive to light: R_f (8% CH_3OH in CH_2Cl_2) 0.7; IR ($CHCl_3$) 3400, 3000, 1680, 1605 cm^{-1}; UV λ_{max} (EtOH) 257 μm (15900), 288 (s, 10800); ^1H NMR δ 0.91, 0.92 (3 H, 2 overlapping t, J = 7.2 Hz, CH_2CH_3). 2.08, 2.18 (3 H, s, NHAc), 2.48, 2.50 (2 H, 2 overlapping q, J = 7.2 Hz, CH_2CH_3), 6.46–7.49 (m, 13 H, including AB q at 6.70, 6.47, J_{AB} = 8.6 Hz, C_6H_4OH). Anal. Calcd for $C_{24}H_{23}NO_2$: C, 80.67; H, 6.44; N, 3.92. Found: C, 80.72; N, 6.30; N, 3.81. Mass spectrum m/e 357 (M^+, 100%), 342 (M – CH_3, 70%), 328 (M – C_2H_5, 12%), 315 (M – CH_2CO, 15%), 300 (M – CH_2CO – CH_3, 37%), 249 (M – CH_2-C_6H_4OH, 25%), 223 (M – C_6H_4NHAc, 29%), 208 (5%), 200 (30%).

1-[4-(Acetylamino)phenyl]-1-[4-[2-(*N*,*N*-dimethylamino)ethoxy]phenyl]-2-phenyl-1-butene (10). Alcohol **9** (1.2 g, 3.4 mM) and 0.6 g (10.7 mM) of KOH were dissolved in 30 mL of ethanol. After 10 min 1.05 g (7.3 mM) of 2-(dimethylamino)ethyl chloride hydrochloride were added in 100 mL of toluene, and the suspension was refluxed for 2 h. Water was added and the solution extracted with ether and worked up as usual. Evaporation gave an oily solid. Chromatography gave a pale yellow waxy solid that could not be obtained crystalline from benzene–hexane or cyclohexane. By NMR it is a 55:45 E:Z ratio. The compound is stable to light: 1.1 g (76% yield); R_f (15% CH_3OH in CH_2Cl_2) 0.4; UV λ_{max} (EtOH) 257 μm (16500), 285 (s,

11 600); ^{1}H NMR δ 0.92, 0.93 (3 H, 2 overlapping t, J = 7.2 Hz, CH_2CH_3), 2.08 (3 H, s, NHAc, E), 2.18 (9 H, s, NHAc, Z), 2.28 (6 H, s, $N(CH_3)_2$, Z), 2.35 (6 H, s, $N(CH_3)_2$, E), 2.48 (2 H, 2 overlapping q, J = 7.2 Hz, CH_2CH_3), double irradiation at 0.9 ppm gave doublet with higher field singlet (Z) higher in intensity, 2.64 (2 H, t, J = 5.9 Hz, OCH_2CH_2N, Z), 2.75 (2 H, t, J = 5.9 Hz, OCH_2CH_2N, E), 3.92 (2 H, t, J = 5.9 Hz, OCH_2, Z), 4.08 (2 H, t, J = 5.9 Hz, OCH_2, E), 6.53–7.49 (m, 13 H, including 8-line 2AB q at 6.9–6.53). Anal. Calcd for $C_{28}H_{32}N_2O_2$: C, 78.50; H, 7.47; N, 6.54. Found: C, 78.39; H, 7.51; N, 6.42. Mass spectrum m/e 428 (M^+, 60%), 370 (M − 58, 7%), 356 (M − 72, 24%), 342 (M − 58 − CO, 57%), 323 (7) 183 (6), 169 (8), 151 (16), 72 (>N^+—C=, 100%), 58 (>N^+=, 78%).

1-(4-Aminophenyl)-1-[4-[2-(*N*,*N*-dimethylamino)ethoxy]phenyl]-2-phenyl-1-butene (11). (Aminoacetyl)tamoxifen (10) (1 g, 2.3 mM) was dissolved in 20 mL of CH_3OH, and 4 N HCl (20 mL H_2O + 10 mL concentrated HCl) was added. The solution was heated at 120 °C for 1.5 h cooled, and made basic. Extraction with CH_2Cl_2 and workup as usual gave a pale yellow viscous oil. Chromatography on silica gel gave pale oily solid, which could not be induced to crystallize; 0.45 g (50% yield). It is stable in the dark but turns dark in light, especially on silica gel: UV λ_{max} (EtOH) 228 μm (17 900), 253 (16 800), 294 (11 400), 340 (s, 5200); ^{1}H NMR δ 0.90 (3 H, 2 overlapping t, CH_2CH_3), 2.31 (6 H, s, $N(CH_3)_2$, Z), 2.37 (6 H, s, $N(CH_3)_2$, E), 2.47 (2 H, 2 overlapping q, CH_2CH_3), 2.67 (2 H, t, J = 6.0 Hz, OCH_2CH_2N, Z), 2.77 (2 H, t, J = 6.0 Hz, OCH_2CH_2N, E), 3.94 (2 H, t, J = 6.0 Hz, OCH_2CH_2N, Z), 4.10 (2 H, t, J = 6.0 Hz, OCH_2CH_2N, E), 6.32–7.40 (13 H, m); isomer ratio 55:45 Z:E. Anal. Calcd for $C_{26}H_{30}N_2O$: C, 80.83; H, 7.77; N, 7.25. Found: C, 80.62; H, 7.69; N, 7.17. Mass spectrum m/e 386 (M^+, 18%), 358 (M − CO, 39%), 342 (M − 44, 100%), 150 (30%), 98 (63%), 72 (>N^+—C=, 58%), 58 (>N^+=, 54%).

4-(Piperidinoazo)-4′-methoxybenzophenone (12). Acetylamino 7 (1.3 g, 5 mM) was hydrolyzed with 20 mL of 6 N HCl by refluxing for 1 h. The clear solution was evaporated to dryness, and 20 mL of H_2O and 1 mL of concentrated HCl were added. The solution was cooled with ice and 0.4 g $NaNO_2$ added in portions. After 20 min of stirring in the cold, it was filtered from some suspended solid and 1 mL of piperidine added. After 0.5 h of stirring at room temperature, the reaction was made alkaline and worked up to give viscous red oil. Chromatography gave a pale yellow solid: 0.8 g (51%; from 7); mp −76 °C; R_f (9:1 CH_2Cl_2–CH_3CH) 0.8; IR ($CHCl_3$) 2960, 1645, 1600 cm^{-1}; UV λ_{max} (EtOH) 226 μm (15 500), 300 (s, 17 850), 345 (25 000); ^{1}H NMR δ 1.73 (6 H, Br s), 3.85 (4 H, br s), piperidino protons, 3.88 (3 H, s, OCH_3), 6.97, 7.82 (4 H, AB q, J = 9.0 Hz, aromatic triazene ring), 7.50, 7.78 (4 H, AB q, J = 8.6 Hz, aromatic OCH_3 ring). Anal. Calcd for $C_{19}H_{21}N_3$: C, 70.59; H, 6.50; N, 13.0. Found: C, 70.38; H, 6.55, N, 13.10.

4-Fluoro-4′-methoxybenzophenone (1) from 12. A. HF. To 0.4 g (1.1 mM) of triazene 12 in 10 mL of benzene was added 9 mL of 40% HF. The reaction was stirred vigorously at room temperature for 0.5 h and at 50 °C for 10 min. It was poured on ice and worked up. Separation on preparative TLC plates gave 98 mg (37% yield) of white solid, identified as 1 by NMR, IR, TLC, and melting point.

B. HF–Py. To 0.4 g (1.1 mM) of triazene 12 was added 3 mL of pyridine hydrofluoride. After the mixture was stirred 0.5 h at room temperature and 10 min at 50 °C it was worked up as in A; yield 30%.

1-[4-(Piperidinoazo)phenyl]-1-[4-[2-(*N*,*N*-dimethylamino)ethoxy]phenyl]-2-phenyl-1-butene (13). To 0.8 g (2 mM) of aminotamoxifen (11) in 50 mL of 6 N HCl, cooled with ice, was added 0.2 g of $NaNO_2$. After 40 min of stirring in the cold, it was filtered and 0.6 mL of piperidine added. The yellow reaction product was stirred for 1 h at room temperature, made basic, and worked up. Chromatography gave a pale yellow viscous solid: 0.7 g (69%); IR ($CHCl_3$) 2960, 1695 cm^{-1}; UV λ_{max} (EtOH) 254 μm (15 400), 310 (s, 14 750), 352 (16 400); ^{1}H NMR δ 0.90 (3 H, 2 overlapping t, J = 7.3 Hz, CH_2CH_3), 1.76 (6 H, narrow m, piperidine protons), 2.34 (6 H, s, $N(CH_3)_2$, Z), 2.38 (6 H, s, $N(CH_3)_2$, E) 2.45 (2 H, 2 overlapping q, J = 7.3 Hz, CH_2CH_3), 2.63 (2 H, t, J = 5.8 Hz, OCH_2CH_2N, Z), 2.76 (2 H, t, J = 5.8 Hz, OCH_2CH_2N, E), 3.80 (4 H, m, $(CH_2)_2N$ piperidino protons), 3.96 (2 H, t, J = 5.8 Hz, OCH_2, Z), 4.11 (2 H, t, J = 5.8 Hz, OCH_2, E), 6.30–7.68 (13 H, m); 1:1 E:Z ratio. Anal. Calcd for $C_{31}H_{38}N_4O$: C, 77.17; H, 7.88; N, 11.62. Found: C, 77.29; H, 7.95; N, 11.54.

Fluorotamoxifen (4) from Triazene 13. Similar to the reaction of the benzophenone triazene 12 the tamoxifen triazene 13 was reacted with 40% HF and pyridine hydrofluoride to yield fluorotamoxifen (4) in 35% and 28% yields, respectively (isolated on preparative TLC, identified by NMR, IR, and TLC).

Cytosol Preparation for Receptor-Binding Studies. Dextran-coated charcoal was used to measure the number of estrogen receptors and the dissociation constants of the test compounds, in rat uterine cytosol: 19-day-old immature rats of the Hebrew University "Sabra" strain were killed by cervical dislocation and their uteri rapidly removed, stripped off adhering fat, and immediately immersed in liquid nitrogen. The uteri were stored at −70 °C and used within 2 months.

For cytosol preparation, uteri were homogenized in TEG buffer (Tris-HCl 0.01 M; EDTA 0.002 M, pH 7.4, 10% glycerol) containing 0.01 M dithiothreitol at 0 °C, using a motor-driven glass–glass homogenizer. The homogenate was centrifuged at 6000g for 10 min (Beckman L3-50, rotor 40) and the resulting supernatant recentrifuged at 105000g for 30 min (Beckman L3-50, rotor 50). The resulting supernatant, containing about 4 mg of protein/mL (about three uteri extracted per milliliter) was immediately frozen in liquid nitrogen and used within 4 months. Just prior to each assay the cytosol fraction used was brought up to 2 uteri/mL, using TEG buffer. Cytosol protein was measured by the Lowry method.[40]

Estimating the number of estradiol receptors in the cytosol fraction and the competitive inhibition of the test materials were evaluated by the Dextran-coated charcoal technique as described Jordan et al.[41] with the following modifications: the charcoal concentration used was 2.5%, and aliquots were spun twice for 10 min, for separating the charcoal and the free radioactive ligand complex, in a Sorvall RC-3 centrifuge, at 8000g. The 200-μL aliquots of cytosol preparations were incubated with 10 mM [3H]estradiol (total binding) or [^{3}H]estradiol plus increasing concentrations of the test compounds for 20 h, at 0 °C. The quantity of [^{3}H]estradiol bound was determined by a charcoal assay. Nonspecific binding was estimated by incubation with 10 000-fold excess of the corresponding test compound and was subtracted from each pair of aliquots in order to obtain the specific binding.

For the saturation analysis of [^{3}H]estradiol binding to rat uterine cytosol, 200-μL aliquots of the cytosol preparation were incubated with increasing concentrations of [^{3}H]estradiol at two different ranges: 1–160 nM (Figure 3 (left)) or 1–40 nM (Figure 3 (right)) for total binding and [^{3}H]estradiol plus DES (2×10^{-5} M) for nonspecific binding. Data were plotted according to Scatchard[42] and corrected according to Rosenthal[43] and Feldman.[44]

After the existence of two types of binding sites (types I and II) were reconfirmed, the K_A values of the test compounds were calculated from the formula

$$K_A = K_L A_{50}/([L^*] + K_L)$$

where K_L is the K_d of the labeled ligand ([^{3}H]estradiol), [L*] is the concentration of free L* in the incubate (10 nM in our experiment), and A_{50} is the concentration of the cold (competing) ligand that causes 50% inhibition of specific binding of [^{3}H]estradiol.

Uterotopic Assay in "Sabra" Rats. Tamoxifen, aminotamoxifen, fluorotamoxifen, and three of their chemical intermediates, alone and in combination with estradiol or hexestrol, were examined for their estrogenic/antiestrogenic activity in 19–21-day-old immature female "Sabra" rats, rats were randomized 7–9 per group, and each compound was injected separately, sc, on three consecutive days. On the fourth day, the animals were killed by

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cervical dislocation and their uteri were removed, stripped of adhering fat, and weighed.

Stock solutions of estradiol (600 μg/mL) and hexestrol (540 μg/d) were prepared in absoluite ethanol and diluted to the desired concentration with saline. Daily doses of estradiol were 0.08, 0.10, 0.16, or 0.20 μg/0.1 mL or 10.0 μg/0.4 mL. Daily doses of hexestrol were 0.05 or 0.5 μg/0.1 mL. Tamoxifen, aminotamoxifen, and fluorotamoxifen were dissolved in propylene glycol.

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Registry No. 1, 345-89-1; (*E*)-**2**, 97732-60-0; (*Z*)-**2**, 97732-61-1; **3**, 97732-62-2; **4**, 97749-43-4; **5**, 1151-94-6; **6**, 4834-72-4; **7**, 97732-63-3; **8**, 97732-64-4; **9**, 97732-65-5; **10**, 97732-66-6; **11**, 97732-67-7; **12**, 97732-68-8; **13**, 97732-69-9; $CH_3CH_2CH(Br)Ph$, 2114-36-5; tamoxifen, 10540-29-1; estradiol, 50-28-2; hexestrol, 84-16-2; diethylstilbestrol, 56-53-1.

Supplementary Material Available: Tables of thermal and positional parameters (3 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Properties of (Carboxyalkyl)amino-Substituted Bicyclic Lactam Inhibitors of Angiotensin Converting Enzyme

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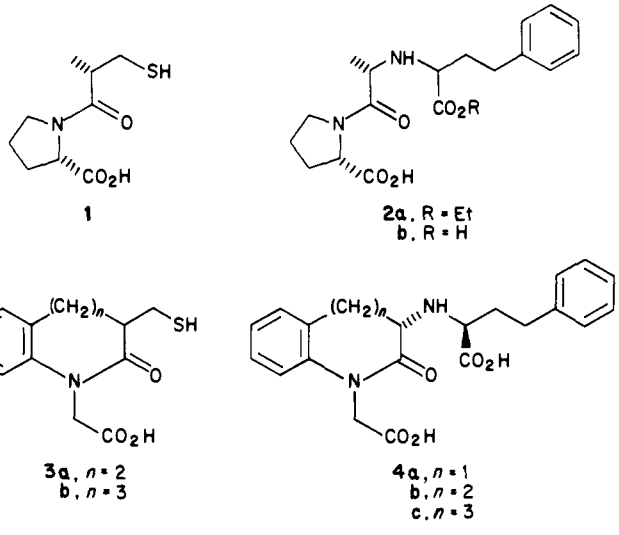
Syntheses of the potent angiotensin converting enzyme inhibitor (3*S*)-1-(carboxymethyl)-3-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-1*H*-[1]benzazepin-2-one (**4b**; CGS 14831) and the related monoester prodrug (**17a**; CGS 14824A) are described together with preparative details for six- and eight-membered ring analogues. Inhibitory potencies and in vivo biological activity of the compounds are discussed. The data indicate that **17a** has a biological profile comparable to that of enalapril.

Captopril (**1**), the first orally effective angiotensin converting enzyme (ACE) inhibitor, is marketed as an antihypertensive and as an agent for the treatment of congestive heart failure.[1] However, there has been concern about the incidence of side effects associated with captopril therapy,[1] and the suggestion has been made that the mercapto function might be a contributory factor.[1] More recent studies have indicated that captopril is effective at lower doses than originally specified[2] and that, under these circumstances, side effects are effectively restricted to a small subgroup of patients having serious complications prior to initiation of the therapy. Studies reported on the clinical application of the non-thiol inhibitor enalapril (**2a**) are not as extensive as those with captopril, but indications are that the incidence of side effects is low.[3]



In a recent paper,[4] the synthesis of the ACE inhibitors (**3a,b**) was described. We considered it desirable to extend these studies to the synthesis of analogues of **3**, incorporating structural features of the prodrug **2a** and the corresponding enzyme inhibitor **2b**.

Chemistry. In order to assess the effect of ring size on the biological activity of the bicyclic lactams, we decided to prepare **4a-c**.

The synthesis of **4a** is outlined in Scheme I. Reductive alkylation of amino lactam **5**[5] with benzylpyruvic acid[6] gave amino acid **6** as a mixture of diastereomers. This mixture was esterified and then alkylated to give diester **8**. Chromatography of **8** gave the individual diastereomers that were separately hydrolyzed to the diacids **4a,d**.

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