Antiestrogen Basicity–Activity Relationships: A Comparison of the Estrogen Receptor Binding and Antiuterotrophic Potencies of Several Analogues of (Z)-1,2-Diphenyl-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-butene (Tamoxifen, Nolvadex) Having Altered Basicity

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A series of N-substituted (Z)-1,2-diphenyl-1-[4-(2-aminoethoxy)phenyl]-1-butenes, analogues of the antiestrogen tamoxifen (Nolvadex), in which the side-chain basicity is varied over a wide range, has been prepared to probe the importance of basicity in evoking estrogen antagonism. All of the compounds, except the pyrrole analogue 14, were found to possess significant antiestrogenic activity in the rat, as measured by their ability to inhibit estrogen-induced uterine growth. This implies that in the tamoxifen series the level of side-chain basicity, at least in the Lowry–Brønsted sense, is not a determining factor in the estrogen antagonist potencies of these compounds.

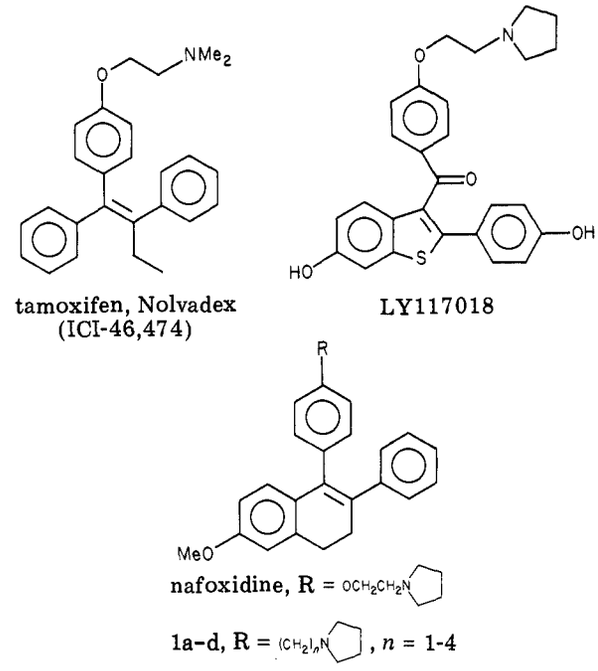
Antiestrogens are a class of compounds which block, at least in part, the action of estrogens in target tissues. They appear to exert their biological effects through their interaction with the estrogen receptor. Interest in antiestrogens has been increased in recent years by the finding that they are effective in controlling several neoplastic diseases, especially hormone-dependent breast cancer, and in correcting various endocrine disorders.[1] In addition to their clinical utility, antiestrogens have proved to be interesting molecular probes of the mechanism of action of estrogens in target tissues.[2]

A structural characteristic of most nonsteroidal antiestrogens is the "basic ether" side chain [see tamoxifen, LY117018, and nafoxidine (Chart I)]. Through careful antiestrogen structure–activity studies, Lednicer showed that the basic groups must be "at a given position in space" for the molecule to be an effective estrogen antagonist.[3,4] A cogent demonstration of this fact was the biological activity of a series of nafoxidine analogues, 1, with side chains of varying length (Chart I). Maximum estrogen antagonism, as judged by an antifertility (antiimplantation) assay in the rat, was achieved when n = 3 and 4; the compounds with n = 1 and 3 had a greater than 2000-fold difference in antifertility activity.[4,5] Jordan has also presented studies and arguments stressing the importance of the proper positioning of the basic group in space.[6,7]

We found this almost universal presence of an appropriate basic side chain in antiestrogens intriguing and hypothesized that the basic group may be important in evoking estrogen antagonism due to its ionic interaction with an acidic amino acid residue in the binding site of the estrogen receptor. This could induce an alteration in the structure and properties of the antiestrogen–receptor complex relative to the estrogen–receptor complex, and this structural alteration might underlie the functional differences between complexes of receptor with estrogen agonists and with nonsteroidal antiestrogens that are manifest in the contrasting biological activities of these classes of compounds.[8,9] To probe the importance of side-chain basicity in evoking estrogen antagonism and to continue our studies on the detailed mechanism of action of antiestrogens,[10] we prepared and evaluated the antiuterotrophic activity and the estrogen receptor binding affinity of a series of tamoxifen analogues in which the side-chain basicity was varied over a wide range (Table I).

Chemistry. Compounds **9–13** (Table I, Scheme I) were prepared in high yield by reacting the pure Z isomer[11] of the side-chain bromide **2** with an excess of the appropriate

Chart I



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(1) For reviews of the pharmacological uses of antiestrogens, see Heel, R. C.; Brogden, R. N.; Speight, T. M.; Avery, G. S. *Drugs* **1978**, *16*, 1–24. Henningsen, B.; Linder, F.; Steichele, C., Eds. *Recent Results Cancer Res.* **1980**, *71*. Sutherland, R. L.; Jordan, V. C., Eds. "Non-Steroidal Antiestrogens"; Academic Press, Sydney, 1981.
(2) Katzenellenbogen, B. S.; Bhakoo, H.; Ferguson, E. R.; Lan, N. C.; Tatee, T.; Tsai, T. L.; Katzenellenbogen, J. A. *Recent Prog. Horm. Res.* **1979**, *35*, 259–300. Horwitz, K. B.; McGuire, W. L. In "Breast Cancer: Advances in Research and Treatment"; McGuire, W. L., Ed.; Pleunum: New York, 1978; Volume 2, p 155–204.
(3) Lednicer, D.; Lyster, S. C.; Aspergren, B. D.; Duncan, G. W. *J. Med. Chem.* **1966**, *9*, 172–176.
(4) Lednicer, D.; Lyster, S. C.; Duncan, G. W. *J. Med. Chem.* **1967**, *10*, 78–84.
(5) While the antiimplantation activities of these nafoxidine analogues were strikingly different, their antiuterotrophic activities were not reported.
(6) Jordan, V. C.; Haldeman, B.; Allen, K. E. *Endocrinology* **1981**, *108*, 1353–1361.
(7) Clark, E. R.; Jordan, V. C. *Br. J. Pharmacol.* **1976**, *57*, 487–493.

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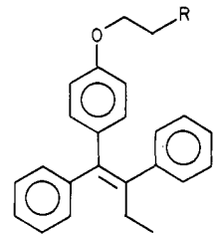
Table I. Tamoxifen Analogues with Altered Basicity

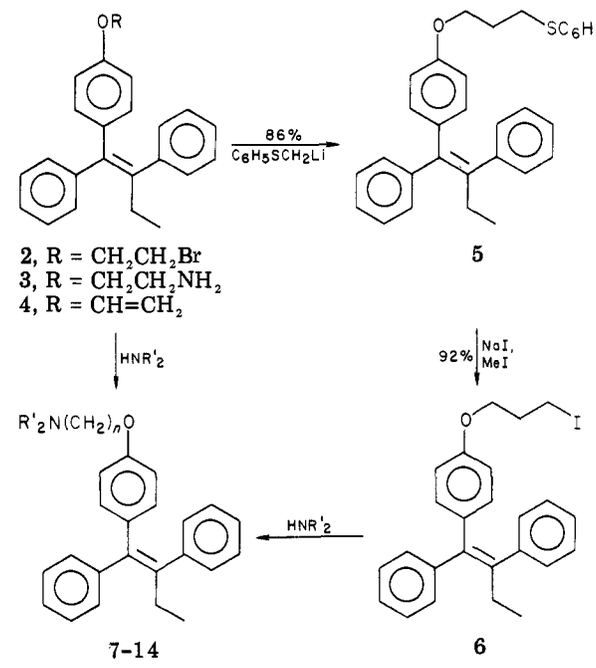


compd	R	rel binding affinity[a] (estradiol = 100%)	approximate pK_a (ref)[b]
7	NHC(=NH)NH_2·HNO_3	5.3	13.65 (15)
8	CH_2-c-NC_4H_8	4.5	11.3 (16)
9	c-NC_4H_8	7.6	11.3 (16)
10	c-NC_5H_{10}	1.1	11.2 (16)
11	$N(CH_2CH_3)_2$·HCl	3.5	10.9 (17)
tamoxifen	$N(CH_3)_2$	2.1	10.8 (17)
12	c-$N(CH_2CH_2)_2N$-CH_3	10.5	9.8[c] (16)
13	c-$N(CH_2CH_2)_2O$	0.2	8.7 (16)
14	c-NC_4H_4	0.1	−3.8 (18)
15	OH	0.9	

[a] All compounds were pure *Z* isomers. The binding affinity for the estrogen receptor was measured in rat uterine cytosol by a competitive binding assay, using [^{3}H]estradiol as a tracer and charcoal dextran as an adsorbant. Under these conditions, estradiol has an affinity (K_D) for the estrogen receptor of 0.3 nM. The binding affinities reported are the average of two measurements and are generally reproducible within ±30%. For details see ref 19. [b] Values reported are for the corresponding secondary amine and are given only to show the basicity trend; the pK_a values have not been determined experimentally. [c] This value is for unsubstituted piperazine.

secondary amine in THF solution. Compound 8 was similarly prepared from the side-chain iodide 6, which was synthesized from bromide 2 by the two-step homologation sequence[12] depicted in Scheme I. The pyrrole analogue 14 was synthesized in 33% yield from 2 by reaction with 1-lithiopyrrole in THF/HMPA (23:1) at −78 °C. The low yield resulted from competing dehydrohalogenation to give the vinyl ether 4 and the formation of other byproducts. Guanidinotamoxifen nitrate 7 was prepared in nearly quantitative yield by reaction of *N,N*-didemethyltamoxifen 3 with 3,5-dimethylpyrazole-1-carboxamidine nitrate in refluxing ethanol.[13] We have previously reported the

Scheme I



syntheses of 2, 3, and the alcohol 15.[14]

Guanidines are among the strongest of the organic bases, and the guanidine analogue 7 would be expected to have a pK_a of approximately 13.7 (Table I). The homologated pyrrolidine compound 8 should be slightly more basic than

(8) Accumulating evidence suggests that complexes of high-affinity antiestrogens with the estrogen receptor are slightly altered relative to complexes of estrogens with the estrogen receptor; see Katzenellenbogen, B. S.; Pavlik, E. J.; Robertson, D. W.; Katzenellenbogen, J. A. *J. Biol. Chem.* **1981**, *256*, 2098–2915. Eckert, R. L.; Katzenellenbogen, B. S. Proceedings of the 63rd Annual Endocrine Society Meeting, Cincinnati, OH, June 1981; Williams & Wilkens: Baltimore, 1981; Abstr 170, p 125.
(9) There have been recent reports in which the binding of antiestrogens to sites other than the estrogen receptor ("antiestrogen specific" sites) has been described. (Sutherland, R. L.; Murphy, L. G.; Foo, M. S.; Green, M. D.; Whybourne, A. M.; and Krazowski, Z. S. *Nature (London)* **1980**, *288*, 273–275. Faye, J.-C.; Lasserre, B.; Bayard, F. *Biochem. Biophys. Res. Commun.* **1980**, *93*, 1225–1231.) However, the concentration of these sites in the immature rat uterus is very low, and the relevance of this binding activity to the antiuterotrophic and to the other antagonistic effects of these antiestrogens remains to be evaluated.
(10) Katzenellenbogen, B. S.; Pavlik, E. J.; Robertson, D. W.; Katzenellenbogen, J. A. *J. Biol. Chem.* **1981**, *256*, 2098–2915. Tatee, T.; Carlson, K. E.; Katzenellenbogen, J. A.; Robertson, D. W.; Katzenellenbogen, B. S. *J. Med. Chem.* **1979**, *22*, 1509–1517. Katzenellenbogen, B. S.; Katzenellenbogen, J. A.; Eckert, R. L.; Hayes, J. R.; Robertson, D. W.; Tatee, T.; Tsai, T. L., *Prog. Cancer Res. Therapy* **1980**, *14*, 309–320.
(11) Only the pure *Z* isomers were prepared and evaluated, since the *E* isomers in the tamoxifen-like compounds are often full estrogen agonists; see ref 6 and references cited therein.
(12) Corey, E. J.; Jautelat, M. *Tetrahedron Lett.* **1968**, 5787–5788.
(13) Scott, F. L.; O'Donovan, D. G.; Reilly, J. *J. Am. Chem. Soc.* **1953**, *75*, 4053–4054.
(14) Robertson, D. W.; Katzenellenbogen, J. A.; Long, D. J.; Rorke, E. A.; Katzenellenbogen, B. S. *J. Steroid Biochem.*, in press.
(15) Smith, P. A. S. "Open-Chain Nitrogen Compounds"; W. A. Benjamin: New York, 1965; Vol 1, p 277.
(16) Albert, A. "Heterocyclic Chemistry"; Athlone Press: Edinburgh, 1968; p 435.
(17) Reference 16, p 20.
(18) Jones, R. A. *Adv. Heterocycl. Chem.* **1970**, *11*, 410.
(19) Hayes, J. R.; Rorke, E. A.; Robertson, D. W.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *Endocrinology* **1981**, *108*, 164–172.

the pyrrolidine compound **9**, since in the former the amine is separated from the electron-withdrawing/inductive effects of the oxygen by an additional methylene unit. Compounds **10–13** in Table I are listed in order of decreasing basicity, with the pK_a values listed being that reported for the secondary amines used in their preparation. Finally, the pyrrole **14** and alcohol **15** would be essentially nonbasic in the Lowry–Brønsted sense.

In this series of antiestrogen analogues, the pK_a of the side chain is varied by over 10 pK_a units. If one assumes that all of the compounds act by binding to the estrogen receptor,[9] then a comparison of their receptor binding affinities and antiestrogenic potency should reveal the importance of side-chain basicity in eliciting estrogen antagonism. For comparative purposes the Lilly antiestrogen LY117018,[20] 6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]-thien-3-yl 4-[2-(1-pyrrolidinyl)ethoxy]phenyl ketone, was also included in these studies.

Biochemical and Biological Characterizations. The binding affinities of these compounds for the estrogen receptor in rat uterine cytosol were determined by a competitive binding assay and are listed in Table I. All of the compounds demonstrated some affinity for the estrogen receptor, and although there was not a direct correlation between binding affinity and side chain basicity, compounds **13–15** (which have the lowest basicity) did exhibit the lowest binding affinities within this series of compounds. The antiestrogen LY117018 had a binding affinity twice that of estradiol.

The estrogen agonist/antagonist properties of these compounds were investigated in the 3-day uterine weight assay in the immature rat; the results are depicted in Figure 1. With the exception of the pyrrole compound **14**, all the tamoxifen analogues exhibited significant antiuterotrophic activities (dashed lines, Figure 1). Compounds **8–13** appeared roughly equivalent to tamoxifen, both in terms of their uterotrophic potencies (solid lines) and in terms of their ability at 50 μg/day to inhibit estradiol-stimulated uterine growth. Even the biological responses elicited by the nonbasic alcohol **15** were not drastically different from those elicited by tamoxifen; both compounds, at 50 μg/day, are able to depress the uterine growth stimulated by 1 μg of estradiol from ca. 400 to 260% that of the control values. This indicates that the antiestrogen side chain basicity, at least in the Lowry–Brønsted sense, is not the most important factor in evoking estrogen antagonism.

The very basic guanidinotamoxifen **7**, at 50 μg/day, has the same antiestrogenic potency as tamoxifen, but unlike tamoxifen it seems not to have reached its maximum antiestrogenic effect, since its inhibition of estrogen-stimulated uterine growth has not reached the level of its own growth stimulation. Thus, at higher doses, it may prove to be a more complete antiestrogen than tamoxifen. Interestingly, the guanidinotamoxifen seems to be less agonistic than tamoxifen at all doses.

The high-affinity antiestrogen LY117018 was the most potent estrogen antagonist in this study (Figure 1), but most remarkable was its very weak estrogen agonism: At 5 μg/day it had reached its maximum uterotrophic potency, stimulating uterine growth to a level ca. 20% that stimulated by 1 μg/day of estradiol; larger doses did not result in an increase in uterine wet weight.

The pyrrole derivative **14**, although capable of binding to the receptor and producing suboptimal estrogen agonism, possesses little, if any, antagonistic activity. Since the

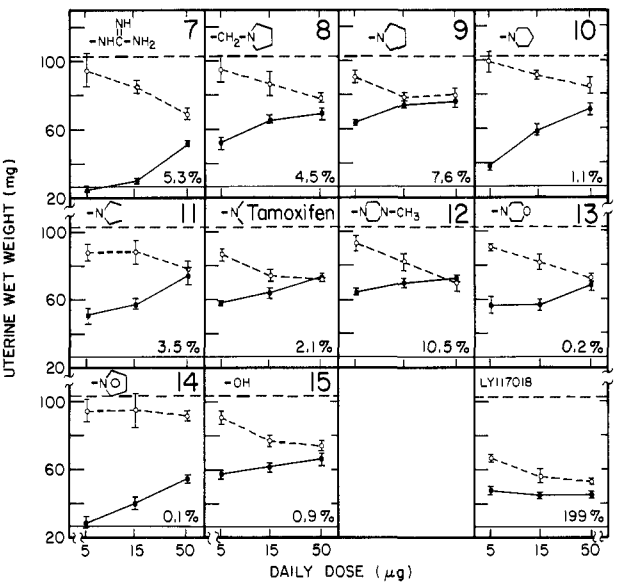


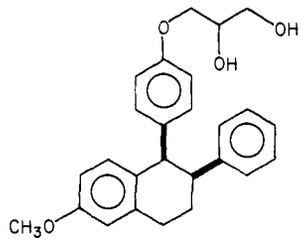
Figure 1. Uterotrophic/antiuterotrophic effects of the tamoxifen analogues with altered basicity. Immature female rats (20–22 days, Holtzman, Madison, WI) were used. Estradiol (1 μg, dashed horizontal line), tamoxifen analogue or LY117018 (5–50 μg, solid line), or both estradiol and compound together (dashed line) were injected sc in oil once daily at 24-h intervals for 3 days, and uterine wet weight was determined 24 h after the third injection. The control (oil vehicle) uterine weights are shown as the solid horizontal line. Values represent the mean ± SEM of four uteri. The number in the lower right corner of each panel represents the binding affinity of each compound for the rat uterine estrogen receptor, expressed relative to that of estradiol (100%) (see also Table I).

nitrogen lone-pair electrons in pyrrole are part of the aromatic π system, they are unavailable for hydrogen bonding. Thus, the observation that all the compounds except the pyrrole exhibit significant antiestrogenic effects may indicate simply that only the availability of a lone pair of electrons for hydrogen bonding is important for evoking estrogen antagonism and that the absolute level of basicity of the side chain is not important. Consequently, the previously described hypothesis than an *ionic interaction* is responsible for inducing an alteration in the antiestrogen–receptor complex (which ultimately translates into estrogen antagonism) is not supported by this study, but a related hypothesis, in which the alterations in receptor structure are the result of a *hydrogen bond interaction* between a binding site amino acid (hydrogen bond donor) and the antiestrogen side chain (hydrogen bond acceptor: –OH or $-NR_2$), may still be plausible. This would account for the ability of all known nonsteroidal antiestrogens, including the compounds not possessing basic side chains such as **14** and (±)-*cis*-3-[4-(1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-1,2-propanediol (U-23469),[21] to act as estrogen antagonists.[22] Further studies must be conducted before this hypothetical mechanism of estrogen antagonism can be proved or disproved.

(20) Black, L. J.; Goode, R. L. *Life Sci.* **1980**, *26*, 1453–1458.

(21) Lednicer, D.; Emmert, D. E.; Lyster, S. C.; Duncan, G. W. *J. Med. Chem.* **1969**, *12*, 881–885.

(22) On the basis of studies of basic ether derivatives of stilbene and bibenzyl compounds (Emmens, C. W.; Collins, D. J.; Hobbs, J. J.; Miller, B. G.; Owen, W. H. *J. Pharm. Exp. Ther.* **1969**, *165*, 52–59), Emmens had suggested that polarity, rather than basicity, was an essential feature of the side chain of antiestrogens. This suggestion was made on the supposition that the side chain was important in controlling the pharmacokinetic behavior of these compounds, rather than their receptor interaction.



U-23469

Experimental Section

Chemical Materials. The pure *Z* isomers of compounds **2**, **3**, and **15** were prepared as described elsewhere.[14] LY117018 was supplied by Eli Lilly and *trans*-tamoxifen was supplied by ICI Ltd. (Macclesfield, United Kingdom) or by Stuart Pharmaceuticals (ICI America). Other chemicals and solvents were purchased from commercial sources and were of analytical reagent grade or better.

Solvents and reagents were used as purchased, except that tetrahydrofuran (THF) was dried immediately before use by distillation from sodium and benzophenone, and dimethylformamide (DMF) and hexamethylphosphoric triamide (HMPA) were dried by vacuum distillation from calcium hydride, purged free of oxygen with argon, and then stored over 4 Å molecular sieves.

Instrumentation and Chemical Procedures. Analytical thin-layer chromatography was performed using 0.20-mm silica gel plastic-backed plates (precoated TLC sheets, silica gel 60 F254, Merck catalog no. 5775), and compounds were visualized by ultraviolet light (254 nm). Preparative thin-layer chromatography was carried out using 2-mm glass-backed silica gel plates (precoated TLC plates, silica gel F-254, Merck). Column chromatography was performed using 0.05–0.20 mm silica gel (Brinkman).

Melting points were determined on a Fisher-Johns melting point apparatus and are not corrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained at 360 MHz on a Nicolet NT-360 spectrometer. Chemical shifts are reported in the δ system of units relative to tetramethylsilane as an internal standard. Mass spectra were obtained on a Varian MAT Model CH-5 mass spectrometer. Microanalytical data were provided by the Microanalytical Service Laboratory of the University of Illinois School of Chemical Sciences.

Except where mentioned otherwise, a standard procedure was used for product isolations; this involved quenching by addition to water, exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. The particular solvents, aqueous washes (if used), and drying agents are mentioned in parentheses after the phrase "product isolation".

(*Z*)-1,2-Diphenyl-1-[4-[3-(phenylsulfenyl)propoxy]phenyl]-1-butene (5). 1,4-Diazabicyclo[2.2.2]octane (420 mg, 3.74 mmol) was dried by azeotrophic distillation with benzene and dissolved in 20 mL of THF. Thioanisole (529 mg, 4.26 mmol) was added, the solution was cooled to 0 °C, *n*-butyllithium (1.6 mL of a 2.04 M solution in hexane, 3.26 mmol) was added dropwise, and then the reaction was allowed to warm to room temperature. After 1.5 h, the yellow organolithium solution was cooled to −78 °C and the bromo compound **2**[14] (500 mg, 1.22 mmol) in 10 mL of THF was added rapidly. The reaction was allowed to warm to room temperature, and after 3 h product isolation (ether, 0.1 N hydrochloric acid, water, brine, magnesium sulfate), column chromatography (40 g of silica gel using a 0–5% ether in hexane gradient), and crystallization of the resulting oil from ethanol/methanol afforded the product as white crystals: yield 470 mg (86%); mp 59–60 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.5 Hz, CH_2CH_3), 2.05 (m, 2, J = 6 Hz, $OCH_2CH_2CH_2S$), 2.45 (q, 2, J = 7.5 Hz, CH_2CH_3), 3.10 (t, 2, J = 6 Hz, $OCH_2CH_2CH_2S$), 4.02 (t, 2, J = 6 Hz, OCH_2CH_2), 6.50 (d, 2, J = 8.64 Hz, Ar H ortho to −OR), 6.77 (d, 2, J = 8.64 Hz, Ar H meta to −OR), 7.10–7.34 (m, 15, Ar H); mass spectrum (70 eV), m/e (relative intensity) 450 (M^+, 24). Anal. ($C_{31}H_{30}OS$) C, H, S.

(*Z*)-1,2-Diphenyl-1-[4-(3-iodopropoxy)phenyl]-1-butene (6). A mixture of the sulfide **5** (430 mg, 0.954 mmol), methyl iodide (11.4 g, 80.31 mmol), sodium iodide (1.5 g, 10 mmol), and 40 mL of DMF was heated in a pressure bottle, with stirring at 80 °C for 24 h. Cooling, product isolation (ether, water, brine, magnesium sulfate), and crystallization from hexane gave 410 mg (92%) of product as slightly yellow crystals, mp 103–106 °C. Recrystallization from hexane afforded the analytical sample as small white needles, mp 106.5–108.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.5 Hz, CH_2CH_3), 2.19. (m, 2, J = 6 Hz, $OCH_2CH_2CH_2I$), 2.45 (q, 2, J = 7.5 Hz, CH_2CH_3), 3.30 (t, 2, J = 6.5 Hz, CH_2I), 3.89 (t, 2, J = 5.7 Hz, CH_2OAr), 6.54 (d, 2, J = 8.64 Hz, Ar H ortho to −OR), 6.77 (d, 2, J = 8.64 Hz, Ar H meta to −OR), 7.13–7.34 (m, 10, Ar H); mass spectrum (70 eV), m/e (relative intensity) 468 (M^+, 100). Anal. ($C_{25}H_{25}IO$) C, H, I.

(*Z*)-1,2-Diphenyl-1-[4-(guanidinoethoxy)phenyl]-1-butene Nitrate (7, Guanidinotamoxifen). A solution of the side-chain amine **3**[14] (342 mg, 1.0 mmol) and 3,5-dimethylpyrazole-1-carboxamidine nitrate (100 mg, 0.497 mmol) in 3 mL of ethanol was heated to reflux for 2.75 h and then cooled. Removal of the solvent in vacuo and recrystallization of the residue from 2-propanol/hexane gave the product **7** (220 mg, 99%) as small plates: mp 161–164 °C; IR (KBr) 1665, 1390 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 2.45 (q, 2, J = 7.56 Hz, CH_2CH_3), 3.49 (t, 2, J = 4.68 Hz, OCH_2CH_2N), 3.96 (t, 2, J = 4.68 Hz, OCH_2CH_2N), 6.50 (d, 2, J = 8.64 Hz, Ar H ortho to −OR), 6.79 (d, 2, J = 8.64 Hz, Ar H meta to −OR), 7.10–7.34 (m, 10, Ar H); mass spectrum (10 eV), m/e (relative intensity) 343 (80), 300 (100), 285 (23). Anal. ($C_{25}H_{28}N_4O_4$) C, H, N.

(*Z*)-1,2-Diphenyl-1-[4-[3-(*N*-pyrrolidino)propoxy]phenyl]-1-butene (8). A solution of iodo compound **6** (150 mg, 0.320 mmol) and pyrrolidine (2.13 g, 29.9 mmol) in 15 mL of THF was refluxed for 4 h. Cooling to room temperature, product isolation (ether, water, brine, sodium sulfate), and crystallization from hexane gave the product as small white plates: yield 102 mg (78%); mp 94.5–95.0 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 1.77 (br s, 4, ring CH_2 nonadjacent to N), 1.92 (m, 2, $CH_2CH_2CH_2$), 2.42–2.58 (m, 8, ring CH_2 adjacent to N, CH_2CH_3, $CH_2CH_2CH_2N$), 3.88 (t, 2, J = 6.48 Hz, OCH_2), 6.54 (d, 2, J = 8.64 Hz, Ar H ortho to −OR), 6.75 (d, 2, J = 8.64 Hz, Ar H meta to −OR), 7.11–7.36 (m, 10, Ar H); mass spectrum (10 eV), m/e (relative intensity) 411 (M^+, 32). Anal. ($C_{29}H_{33}NO$) C, H, N.

(*Z*)-1,2-Diphenyl-1-[4-[2-(*N*-pyrrolidino)ethoxy]phenyl]-1-butene (9). This compound was prepared in 89% yield according to the procedure used for the synthesis of **8**, using bromo compound **2**[14] and pyrrolidine. The product was obtained as white, flocculent crystals from hexane (−25 °C): mp 89.5–90.5 °C (lit.[23] mp 86–88 °C); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 1.77 [br s, 4, NCH_2CH_2 (ring)], 2.45 (q, 2, J = 7.56 Hz, CH_2CH_3), 2.56 [br s, 4, NCH_2 (ring)], 2.80 (t, 2, J = 6.12 Hz, OCH_2CH_2N), 3.96 (t, 2, J = 6.12 Hz, OCH_2CH_2N), 6.55 (d, 2, J = 8.64 Hz, Ar H ortho to −OR), 6.76 (d, 2, J = 8.64 Hz, Ar H meta to −OR), 7.10–7.36 (m, 10, Ar H); mass spectrum (70 eV), m/e (relative intensity) 397 (M^+, 5). Anal. ($C_{28}H_{31}NO$) C, H, N.

(*Z*)-1,2-Diphenyl-1-[4-[2-(*N*-piperidino)ethoxy]phenyl]-1-butene (10). This compound was prepared in 94% yield according to the procedure used for the synthesis of **8**, using bromo compound **2**[14] and piperidine. The product was obtained as small white needles from hexane: mp 113–114 °C (lit.[23] mp 110–112 °C); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 1.41–1.65 (m, 6, ring CH_2 nonadjacent to N), 2.44 (m, 6, ring CH_2 adjacent to N and CH_2CH_3), 2.68 (t, 2, J = 6.12 Hz, OCH_2CH_2N), 3.96 (t, 2, J = 6.12 Hz, OCH_2CH_2N), 6.54 (d, 2 J = 8.64 Hz, Ar H ortho to −OR), 6.76 (d, 2, J = 8.64 Hz, Ar H meta to −OR), 7.10–7.34 (m, 10, Ar H); mass spectrum (10 eV), m/e (relative intensity) 411 (M^+, 12). Anal. ($C_{29}H_{33}NO$) C, H, N.

(*Z*)-1-[4-[2-(Diethylamino)ethoxy]phenyl]-1,2-diphenyl-1-butene Hydrochloride (11). This compound was prepared in 85% yield according to the procedure used for the synthesis of **8**, using bromo compound **2**[14] and diethylamine; the product was isolated as the hydrochloride salt which, after recrystallization from isopropyl alcohol/ether, gave large white crystals: mp 200–201.5 °C (with prior softening); ^{1}H NMR (Me_2SO-d_6) δ 0.84 (t, 3, J = 7.56 Hz, CH_2CH_3), 1.19 (t, 6, J = 6.84 Hz, NCH_2CH_3), 2.36 (q, 2, J = 7.56 Hz, CH_2CH_3), 3.14 (m, 4, NCH_2CH_3), 3.38 (t, 2 J = 4.68 Hz, OCH_2CH_2N), 4.21 (t, 2, J = 4.68 Hz, OCH_2CH_2N),

(23) Harper, M. J. K.; Richardson, D. N.; Walpole, A. L.; British Patent 1 064 629, 1967.

6.65 (d, 2, J = 8.64 Hz, Ar H ortho to -OR), 6.76 (d, 2, J = 8.64 Hz, Ar H meta to -OR), 7.11-7.37 (m, 10, Ar H); mass spectrum (10 eV), m/e (relative intensity) 399 (M^+, 5). Anal. ($C_{28}H_{34}ClNO$) C, H, N.

(*Z*)-1,2-Diphenyl-1-[4-[2-(*N*-methylpiperazino)ethoxy]phenyl]-1-butene (12). This compound was prepared in 94% yield according to the procedure used for the synthesis of 8, using bromo compound **2**[14] and *N*-methylpiperazine. The product was obtained as flocculent white needles from hexane: mp 109-110.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 2.27 (s, 3, NCH_3), 2.39-2.70 (br m, 8, ring CH_2), 2.45 (q, 2, J = 7.56 Hz, CH_2CH_3), 2.73 (t, 2, J = 5.76 Hz, OCH_2CH_2N), 3.96 (t, 2, J = 5.76 Hz, OCH_2CH_2N), 6.54 (d, 2, J = 8.64 Hz, Ar H ortho to -OR), 6.76 (d, 2, J = 8.64 Hz, Ar H meta to -OR), 7.10-7.36 (m, 10, Ar H); mass spectrum (10 eV), m/e (relative intensity) 426 (M^+, 7). Anal. ($C_{29}H_{34}N_2O$) C, H, N.

(*Z*)-1,2-Diphenyl-1-[4-[2-(*N*-morpholino)ethoxy]phenyl]-1-butene (13). This compound was prepared in 83% yield according to the procedure used for the synthesis of 8, using bromo compound **2**[14] and morpholine. The product was obtained as colorless plates from hexane containing a trace of THF: mp 131.5-133 °C (lit.[23] mp 130-132 °C); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 2.45 (q, 2, J = 7.56 Hz, CH_2CH_3), 2.52 (t, 4, J = 4.32 Hz, ring CH_2 adjacent to N), 2.71 (t, 2, J = 6.48 Hz, OCH_2CH_2N), 3.70 (t, 4, J = 4.32 Hz, ring CH_2 adjacent to O), 3.97 (t, 2 J = 6.48 Hz, OCH_2CH_2N), 6.54 (d, 2, J = 8.64 Hz, Ar H ortho to -OR), 6.76 (d, 2, J = 8.64 Hz, Ar H meta to -OR), 7.11-7.34 (m, 10, Ar H); mass spectrum (10 eV), m/e (relative intensity) 413 (M^+, 28). Anal. ($C_{28}H_{31}NO_2$) C, H, N.

(*Z*)-1,2-Diphenyl-1-[4-[2-(*N*-pyrrolo)ethoxy]phenyl]-1-butene (14). *n*-Butyllithium (3 mL of a 2.44 M solution in hexane, 7.32 mmol) was added dropwise to a stirred solution of pyrrole (600 mg, 8.94 mmol) in 15 mL of THF at 0 °C. After 15 min, 1 mL of HMPA was added, the solution was cooled to -78 °C, and bromo compound **2**[14] (200 mg, 0.491 mmol) in 8 mL of THF was slowly added. The reaction was allowed to warm slowly to room temperature, and after 3 h, product isolation (ether, water, brine, sodium sulfate) and chromatography (one 20 × 20 cm preparative TLC plate; 10% ether in hexane) gave two bands, which were isolated and crystallized from hexane.

The more mobile product gave large prisms (36 mg) with mp 110-111.5 °C whose spectral characteristics identified it as the vinyl ether (**4**) resulting from dehydrohalogenation of the starting material: IR (CCl_4) 1648 cm^{-1} ($OCH{=}CH_2$); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, CH_2CH_3), 2.45 (q, 2, CH_2CH_3), 4.15-4.90 (m, 3, vinyl H), 6.56-7.50 (m, 14, Ar H); mass spectrum (70 eV) m/e (relative intensity) 326 (M^+, 100). Anal. ($C_{24}H_{22}O$) C, H.

The less mobile product gave white flocculent crystals (65 mg, 33%), mp 97.5-98 °C, whose spectral characteristics identified it as the pyrrole analogue of tamoxifen (**14**): IR (CCl_4) 722 cm^{-1} (pyrrole CH); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3, J = 7.56 Hz, CH_2CH_3), 2.45 (q, 2, J = 7.56 Hz, CH_2CH_3), 4.06 and 4.18 (each t, each 2, J = 5.4 Hz, OCH_2CH_2N), 6.12 (m, 2, pyrrole β-H), 6.51 (d, 2, J = 8.64 Hz, Ar H ortho to -OR), 6.70 (m, 2, pyrrole α-H), 6.76 (d, 2, J = 8.64 Hz, Ar H meta to -OR), 7.10-7.34 (m, 10 Ar H); mass spectrum (10 eV), m/e (relative intensity) 393 (M^+, 100). Anal. ($C_{28}H_{27}NO$) C, H, N.

Biochemical and Biological Methods. Complete experimental details for the relative binding affinity and uterotrophic and antiuterotrophic activity determinations can be found in ref 19. A synopsis of these methods is given in the legend to the figure and the table footnotes.

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Lipophilic 5′-(Alkyl phosphate) Esters of 1-β-D-Arabinofuranosylcytosine and Its N^4-Acyl and 2,2′-Anhydro-3′-*O*-acyl Derivatives as Potential Prodrugs

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Lipophilic 5′-(alkyl phosphate) esters of 1-β-D-arabinofuranosylcytosine (*ara*-C) and several N^4-acyl and 3′-*O*-acyl-2,2′-anhydro derivatives of *ara*-C were synthesized as potential prodrugs of *ara*-C 5′-monophosphate (*ara*-CMP). Alkylphosphorylation of *ara*-C, N^4-palmitoyl-*ara*-C, and N^4-stearoyl-*ara*-C was achieved in a single continuous operation by allowing the nucleoside to react with $POCl_3$ in trimethyl or triethyl phosphate and adding the appropriate anhydrous alcohol directly to the intermediate phosphorodichloridate without isolation. Similar reaction of cytidine yielded cytidine 5′-(alkyl phosphate) esters, which on treatment with myristoyl or palmitoyl chloride in the presence of boron trifluoride gave 3′-*O*-acyl-2,2′-anhydro-*ara*-C 5′-(alkyl phosphate) esters. *Ara*-C 5′-(*n*-butyl phosphate) (**1b**), N^4-palmitoyl-*ara*-C 5′-(*n*-butyl phosphate) (**1h**), and 2,2′-anhydro-3′-*O*-palmitoyl-*ara*-C 5′-(*n*-butyl phosphate) (**2h**) were tested against L1210/*ara*-C leukemia in mice in the hope that this kinase-deficient tumor would respond to treatment with these "prephosphorylated" derivatives, but no activity was observed. Of the simple 5′-(alkyl phosphate) esters tested in culture against L1210 leukemic cells, only *ara*-C 5′-(glyceryl phosphate) (**1g**) showed toxicity comparable to *ara*-CMP (ID_{50} = 0.35 and 0.65 μM, respectively), suggesting that β-hydroxyalkyl phosphate esters may be worthwhile to examine further as prodrugs of *ara*-CMP.

Although 1-β-D-arabinofuranosylcytosine (*ara*-C) enjoys widespread use in cancer chemotherapy and is the drug of choice for the treatment of adult myelogenous leukemia,[1] its short plasma half-life and marked schedule dependence require that it be given either in precisely adjusted cycles of intermittent therapy[2] or by continuous infusion[3,4] for maximum benefit. The schedule dependence of *ara*-C results from the fact that its biochemical effect on DNA synthesis is highly S-phase specific,[5] whereas its

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