Synthesis and Structure–Affinity of a Series of 7α-Undecylestradiol Derivatives: A Potential Vector for Therapy and Imaging of Estrogen-Receptor-Positive Cancers

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Received February 16, 1989

A series of 7α-undecylestradiol derivatives, featuring various substituents at the end of the undecyl spacer chain, were synthesized and evaluated for their interaction with the estrogen receptor and nonreceptor sites. Their relative binding affinities (RBA) for calf uterine estrogen receptors were measured by competitive binding assays and varied between 0.5 and 8.4% of that of unlabeled 17β-estradiol. Enhanced lipophilicity and steric hindrance of the substituent on the end of the spacer chain resulted in decreased binding affinity for the estrogen receptor, while interactions with nonreceptor sites increased. RBA values were not affected by prolonged incubation times, suggesting a stable ligand–receptor complex. The potential to use the 7α-undecylestradiol as a vector for site-selective delivery of diagnostic and therapeutic moieties to estrogen-receptor-positive human cancers is discussed.

The presence of estrogen receptors in about 66% of human breast cancers is now well-established.[1,2] About 50–65% of patients with estrogen-receptor-positive breast tumors respond with remission to endocrine therapy[2,3] while only 25% of patients with endometrial cancers[4,5] and very few with ovarian carcinomas[4] will respond to such a treatment. The estrogen receptor can be purified by affinity chromatography and Bucourt et al.[6] reported that estradiol 7α-derivatives attached via a long spacer chain to an insoluble matrix selectively bind estrogen receptors. These authors also pointed out that long spacer chains consisting of 11 carbon atoms provide for the best interaction with the estrogen receptor. On such accounts, we conjectured that the use of a similar spacer chain attached at the 7α-position of estradiol could allow relatively bulky groups to be transported by estradiol in vivo while ensuring minimal interference between the steroid moiety and receptor binding sites. Such an estradiol derivative could in turn serve as a vector to carry cytotoxic agents[8] or γ-emitters[9,10] to the nuclei of target cells rich in estrogen receptor, such as breast tumor cells, providing for a more selective treatment and diagnosis of selected cancers. The rationale for using an estradiol-based vector is based on its binding to the estrogen receptor and consequently its potential to concentrate attached moieties in estrogen-receptor-positive mammary tumor cells. In this paper, we describe the synthesis of a series of 7α-undecylestradiol analogues with different substituents at the end of the spacer chain, including halogens and more bulky lipophilic groups. The effect of these substituents on the interaction

Table I. Relative Binding Affinities of Estradiol Derivatives

	RBA[a]				
compd (R)	2 h	12 h	20 h	20 h (DMF)	RBA (DMF)/RBA[b]
2 (OH)	2.2	2.9	2.0	8.4	4
5 (F)	1.2	1.3	1.5	6.0	4
3 (Cl)	0.7	0.7	0.7	4.1	6
4 (Br)	0.05	0.05	0.2	1.6	8
6 (I)	0.05	0.04	0.1	1.1	11
7 (O-C_6H_5)	0.06	0.1	0.1	2.6	26
8 (O-4-IC_6H_4)	0.08	0.1	0.09	0.5	5
9	0.07	0.09	0.09	0.5	5
16α-iodo-E_2[c]	26.4	22.1	27.1	45.9	2
17α-ethynyl-E_2[c]			100	100	1
hexestrol[c]			37.6	65.4	2
estrone[c]			7.1	15.0	2

[a] The relative binding affinity (RBA) for the calf uterine estrogen receptor is the ratio between the concentrations of unlabeled 17β-estradiol and the competitor required to decrease the amount of bound 17β-[^{3}H]estradiol by 50%. Incubation times varies from 2 to 20 h and the 20-h incubation was repeated in the presence of 7% DMF at 4 °C. [b] The ratio between RBA values obtained after 20 h incubation with and without DMF. [c] These compounds were included as references. E_2 = 17β-estradiol.

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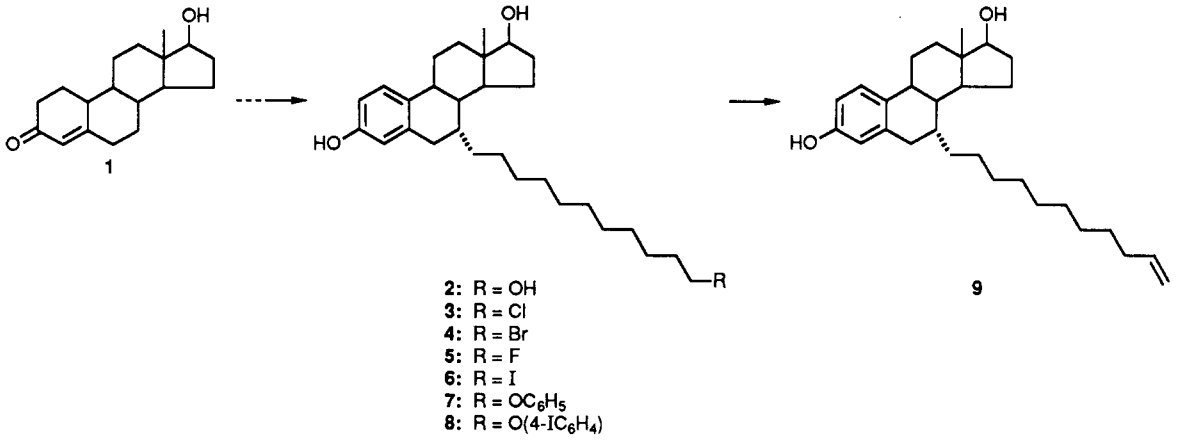
Scheme I



of the 7α-undecylestradiol carrier with the estrogen receptor and nonreceptor binding sites is described and a structure–affinity relationship is put forth.

Chemistry. Hydroxyundecyl derivative **2** was synthesized from 19-nortestosterone (**1**) as previously described[6] (Scheme I). Briefly, **1** is converted to 17β-hydroxyestra-4,6-dien-3-one 17-acetate followed by conjugate addition on the dienic ketone of the undecyl moiety via a Grignard reagent. The bromo and chloroundecyl analogues **4** and **3** were obtained in good yields by selective substitution of the undecyl hydroxy group of triol **2** with CBr_4 or CCl_4, in the presence of triphenylphosphine in dry acetonitrile and THF.[11] In the case of **3**, thermic energy was required to complete the reaction.

Fluoroundecyl analogue **5** was prepared in good yield by substitution of the bromo group of **4** using 4 equiv of n-Bu_4NF in an anhydrous,[12] aprotic solvent. Attempts to increase the yield via protection of the hydroxy groups of **4** with tetrahydropyran from the reaction with dihydropyran/p-toluenesulfonic acid/CH_2Cl_2, prior to n-Bu_4NF treatment,[13] failed and instead resulted in an augmented formation of secondary products. Likewise, conversion of **2** to the triflate derivative followed by selective substitution of the primary triflate with F^- of n-Bu_4NF[14] did not give a better yield of **5**.

Formation of iodoundecyl derivative **6** was accomplished in methyl ethyl ketone by nucleophilic substitution of the bromo group in **4**.[7,9] The phenoxy and (*p*-iodophenoxy)-undecyl derivatives **7** and **8** were prepared by reacting **4** with phenol and *p*-iodophenol in DMF in the presence of $NaHCO_3$.[15] The elimination product **9** was obtained as a minor product during these nucleophilic substitution reactions. All assigned structures were confirmed by UV, ^1H NMR, mass spectroscopy, and combustion analyses.

Binding Affinity for the Calf Uterine Estrogen Receptor. Relative binding affinities (RBA) for calf uterine estrogen receptors of compounds **2–9**, together with a few reference steroids, were measured by competitive binding assays using the dextran-coated-charcoal technique.[7,13,15] Different incubation times were studied to assess the stability of the complexes. The displacement curves of bound 17β-[^{3}H]estradiol by 7α-undecylestradiol derivatives **2–9** (Figure 1) were parallel to the curve obtained with unlabeled 17β-estradiol, suggesting competitive binding with estrogen receptor and a common binding site for all test compounds. The RBA values, representing the ratio between unlabeled 17β-estradiol and competitor concentrations required for 50% competition of specific 17β-[^{3}H]estradiol binding, are reported in Table I.

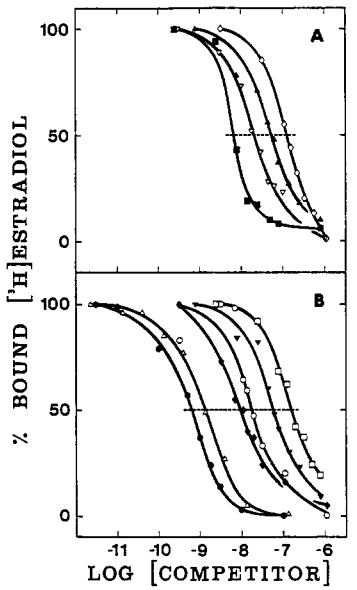


Figure 1. Competitive binding assay of estradiol derivatives (A) **2** (■), **7** (▽), **4** (▲), and **8** (◇); and (B) 16α-iodoestradiol (as an internal standard) (△), **5** (◆), **3** (○), **6** (▼), **9** (□), and unlabeled 17β-estradiol (●) for calf uterine estrogen receptor labeled with 17β-[^{3}H]estradiol (incubation for 20 h at 4 °C in the presence of 7% DMF). The concentration required for 50% competition was used to calculate RBA values (see Table I).

Addition of DMF to the incubation mixture reduces nonspecific binding[16] and this approach was used to assess interaction of the estradiol derivatives with estrogen receptor binding sites. Hydroxyundecyl compound **2** showed the highest affinity for the estrogen receptor with a RBA of 8.4, measured in the presence of DMF after a 20-h incubation time. Under the same conditions, *p*-iodophenoxy and undecane analogues **8** and **9** gave the lowest RBA (0.5) of the series. In the absence of DMF, the RBA

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values ranged from 0.09 (8 and 9) to 2.0 (2), reflecting the extent of nonreceptor binding of the 7α-undecylestradiol derivatives. Prolonged incubation times had little effect on the affinity of the highly lipophilic 7α-undecylestradiols for the estrogen receptor, suggesting the relative stability of the ligand–estrogen receptor complexes.[17]

With the exception of undecene derivative 9, the 7α-undecylestradiol analogues are presented in Table I in order of increasing lipophilicity and steric hindrance of the terminal substituents,[18] e.g., OH < F < Cl < Br < I < OC_6H_5 < OC_6H_4I. The RBA values, measured after 20 h of incubation, decrease with enhanced lipophilicity and bulkiness of the substituent, which is in agreement with observations made by others on hexestrol,[11,19,20] estradiol,[21] and estrogen[22] derivatives. The same correlation is observed in the presence of DMF, e.g. the more lipophilic and bulky the substituent, the lower the affinity for the estrogen receptor. Thus, in the series of halogenated derivatives 3–6, fluoroundecyl derivative 5 exhibited a 15 times higher RBA than the iodo analogue 6. Phenoxyundecyl derivative 7 appears to be an exception to this rule in that its RBA of 2.6 is slightly higher than the RBA of 1.6 measured for bromo analogue 4. The ratios between RBA values obtained after 20 h of incubation with and without DMF reflect the extent of nonreceptor binding of the various estradiol derivatives and are included in Table I. These values again suggest that enhanced lipophilicity and steric interference of the substituent facilitates the interaction with nonspecific binding sites,[23] with (p-iodophenoxy)undecyl 8 as the only exception. The unstable primary C–Br and C–I bonds[7,11,24] in 4 and 6 may contribute to their apparent relative high affinity for nonreceptor sites. The reference products 16α-iodoestradiol, 17α-ethynylestradiol, hexestrol, and estrone (Table I) gave RBA values similar to those earlier reported.[17,19] Their lower affinity for nonreceptor sites, as compared to the 17α-undecylestradiol derivatives, is in line with their more hydrophobic nature.

The relative binding affinities of the various 7α-undecylestradiol derivatives for the combined estrogen receptor and the nonreceptor sites seem to reach a lower threshold of about 0.1 and, in the presence of DMF, 0.5. It is of interest to note that similar RBA values have been reported for clinically used antiestrogen drugs, such as tamoxifen.[25]

Discussion

In this study we have evaluated the potential of a linear, 11-carbon spacer chain, attached onto the 7α-position of 17β-estradiol, to serve as a link between relative bulky substituents and estradiol. Both enucleation[26] and immunocytochemical[27] studies indicate that the estrogen receptor is mainly localized in the nucleus of target cells. In fact, the presence of nuclear estrogen receptor in cytosolic fractions, as used in our binding assays, is believed to be an homogenization artefact.[28] Related work by Bucourt et al.[6] suggested that the use of a long, linear spacer chain would allow attached functional groups to protrude from the receptor–hormone complex, thus rendering this type of carrier as a suitable vector to direct therapeutic and diagnostic moieties to DNA of estrogen-receptor-rich cancer cells.[7] Consequently, given sufficient affinity for the estrogen receptor, the 7α-undecylestradiol could serve as a selective probe in the management of estrogen-receptor-positive human cancers.

We have shown that bromoundecyl 4 is a convenient starting material for the preparation of the analogous fluoro-, iodo-, phenoxy-, and (p-iodophenoxy)undecyl derivatives 5–8. Undecene product 9 is obtained as a secondary product during these nucleophilic substitution reactions. It is evident that in the same manner cytotoxic agents, radiosensitizers, and γ-emitting substituents could be introduced at the end of the spacer chain of 4 to yield functionalized estradiol derivatives.

Increased lipophilicity and steric hindrance of the substituent at the end of the spacer chain resulted in lower affinities for the estrogen receptor and enhanced binding for nonreceptor sites. Substitution of Br in 4 with cytotoxic or γ-emitting moieties will likely produce derivatives with RBA values within the range of observed affinity values (0.5–8.4). The complexes of the 7α-undecylestradiol derivatives 2–9 with the estrogen receptor are of a high stability and they dissociate as slowly as complexes of the naturally occurring 17β-estradiol. This stability is most likely due to the highly lipophilic nature of such derivatives. Thus, although it is not possible to predict whether the relative low RBA values suffice for site-selective drug delivery, the stability of the ligand–receptor complexes will be advantageous to this end. It should also be noted that antiestrogens, such as tamoxifen, which have found clinical applications, exhibit similar estrogen-receptor affinities[25] as those observed with our 7α-undecylestradiol analogues. In order to assess the potential of the 7α-undecylestradiol to serve as a vector to produce the desired therapeutic or diagnostic gain, in vivo pharmacokinetics data of radiolabeled analogues 5, 6, and 8 will be required and studies to this end are in progress in our laboratory.

Experimental Section

Chemistry. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride prior to use. Anhydrous solvents were dried over molecular sieves. Melting points (mp) were taken on a Fisher-Johns apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on 0.25-mm Macherey-Nagel silica gel Polygram Sil G/UV_{254} pre-coated plastic plates, with EtOAc/hexane 4:1 as eluting solvent. High-performance liquid chromatography (HPLC) analyses were carried out on a

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Varian 5000 HPLC utilizing a CSC spherisorb ODS-2 reverse-phase column (C-18, 5 μm, 25 × 0.94 cm; CSC, Montreal, Quebec, Canada) at a flow rate of 2 mL/min. Steroids were detected by their UV absorption at 280 nm with a Varian UV-100 variable-wavelength detector. Ultraviolet spectra (UV) were obtained on a Varian UV/vis 2200 spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 250 MHz on a Bruker WM spectrophotometer using $CDCl_3$ as the solvent. Proton chemical shifts (δ) are reported relative to tetramethylsilane as an internal standard. Low-resolution mass spectra (MS) were determined in the electron impact mode on a Hewlett-Packard 5988A quadrupole spectrometer. The reported data are for an electron energy of 70 eV and are expressed in m/z (intensity relative to base peak = 100). Electron-impact (EI) high-resolution mass spectra (HRMS) were measured on a Micromass V-9 ZAB-1F spectrometer at 70 eV ionization voltage. Elemental analyses were performed by Organic Microanalyses, Montreal, Quebec, Canada and by Guelph Chemical Laboratories Ltd., Guelph, Ontario, Canada.

16α-Iodoestradiol was synthesized accordingly to the procedure of Hochberg;[9] 17α-ethynylestradiol, hexestrol, and estrone were obtained commercially. 7α-(11-Hydroxyundecyl)estra-1,3,5-(10)-triene-3,17β-diol (2) was prepared from 19-nortestosterone (1) by the method of Bucourt et al.[6] with some minor modifications and was crystallized from ether as a white powder (overall yield 8%): mp 75–76 °C; TLC, R_f 0.28; HPLC, t_R = 15 min (MeOH/H_2O 90:10); MS m/z 442 (43, M^+), 424 (3), 271 (10), 253 (12), 171 (23), 157 (100), 145 (54). Anal. (HRMS) Calcd for $C_{29}H_{46}O_3$ 442.3447, found 442.3444. Anal. C, H. The UV and ^{1}H NMR spectra were identical with those reported by Bucourt et al.[6]

7α-(11-Chloroundecyl)estra-1,3,5(10)-triene-3,17β-diol (3). To the triol **2** (80 mg, 0.18 mmol) was added triphenylphosphine (94 mg, 0.36 mmol) in dry carbon tetrachloride (3 mL), dry THF (0.4 mL), and dry acetonitrile (0.8 mL). The mixture was refluxed under N_2 for 1.5 h. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (90 g, silica gel, hexane/EtOAc 9:1) to give compound **3** as a resin. The chloro derivative **3** was further purified by preparative HPLC (MeOH/H_2O 90:10 t_R = 30 min) and crystallized from ether as a white powder (35 mg, 42%): mp 64–65 °C; TLC, R_f 0.52; UV (EtOH) 281, 287 nm; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 1.03–2.35 (br m, 32 H, chain and steroid), 2.67–2.91 (m, 2 H, 6-CH_2), 3.53 (t, 2 H, J = 6.8 Hz, CH_2Cl), 3.75 (t, 1 H, J = 8.3 Hz, 17-H), 4.67 (s, 1 H, ArOH), 6.55 (d, 1 H, J = 2.8 Hz, 4-H), 6.63 (dd, 1 H, J = 8.4, 2.8 Hz, 2-H), 7.15 (d, 1 H, J = 8.7 Hz, 1-H); MS m/z 462 (45, M^+), 460 (100, M^+), 271 (2), 253 (18), 171 (21), 157 (78), 145 (64). Anal. (HRMS) Calcd for $C_{29}H_{45}O_2Cl$ 460.3108, found 46.3113. Anal. C, H, Cl.

7α-(11-Bromoundecyl)estra-1,3,5(10)-triene-3,17β-diol (4). A mixture of triol **2** (30 mg, 0.068 mmol) and carbon tetrabromide (62 mg, 0.19 mmol) in dry acetonitrile (0.8 mL) and dry THF (0.2 mL) was left under N_2 in a 40 °C bath of an ultrasonic apparatus for 2 min to effect dissolution. Triphenylphosphine (72 mg, 0.27 mmol) and dry methylene chloride (0.2 mL) were added, and the solution was stirred at room temperature under N_2 for 1 h. After evaporation of the solvent under reduced pressure, the residue was purified by column chromatography (90 g, silica gel, hexane/EtOAc 9:1) to give compound **4** as a resin. The bromo derivative **4** was further purified by preparative HPLC (methanol/H_2O 90:10, t_R = 35 min) and crystallized from ether as a white powder (33 mg, 95%): mp 61–62 °C; TLC, R_f 0.52; UV (EtOH) 281, 287 nm; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 0.90–2.33 (br m, 32 H, chain and steroid), 2.67–2.91 (m, 2 H, 6-CH_2), 3.40 (t, 2 H, J = 6.9 Hz, CH_2Br), 3.75 (t, 1 H, J = 8.3 Hz, 17-H), 6.55 (d, 1 H, J = 2.8 Hz, 4-H), 6.63 (dd, 1 H, J = 8.4, 2.8 Hz, 2-H), 7.15 (d, 1 H, J = 8.4 Hz, 1-H); MS m/z 506 (51, M^+), 504 (51, M^+), 424 (3), 271 (4), 253 (9), 171 (29), 157 (100), 145 (70). Anal. (HRMS) Calcd for $C_{29}H_{45}O_2Br$ 504.2603, found 504.2606. Anal. C, H, Br.

7α-(11-Fluoroundecyl)estra-1,3,5(10)-triene-3,17β-diol (5). To a solution of **4** (26 mg, 0.051 mmol) dissolved in dry THF (0.4 mL) was added 20 μL (0.20 mmol) of 1 M tetrabutylammonium fluoride in THF. The resulting solution was stirred at 0 °C under N_2 for 15 min. After evaporation of the solvent under reduced pressure, the mixture was dissolved in EtOAc and washed with HCl (10%) and water. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. Fluoro compound **5** was purified by preparative HPLC (MeOH/H_2O 90:10, t_R = 23 min) and crystallized from ether as an off-white powder (5.7 mg, 25%): mp 56–57 °C; TLC, R_f 0.57; UV (EtOH) 280, 287 nm; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 1.00–2.35 (br m, 32 H, chain and steroid), 2.67–2.91 (m, 2 H, 6-CH_2), 3.75 (t, 1 H, J = 8.3 Hz, 17-H), 4.43 (dt, 2 H, J = 47.4, 6.2 Hz, CH_2F), 4.71 (s, 1 H, ArOH), 6.55 (d, 1 H, J = 2.7 Hz, 4-H), 6.63 (dd, 1 H, J = 8.4, 2.8 Hz, 2-H), 7.15 (d, 1 H, J = 8.4 Hz, 1-H); MS m/z 444 (84, M^+), 271 (5), 253 (10), 171 (27), 157 (100), 145 (76). Anal. (HRMS) Calcd for $C_{29}H_{45}O_2F$ 444.3403, found 444.3403. Anal. C, H, F.

7α-(11-Iodoundecyl)estra-1,3,5(10)-triene-3,17β-diol (6). A mixture of **4** (29 mg, 0.057 mmol) and sodium iodide (170 mg, 1.1 mmol) in methyl ethyl ketone (1.8 mL) was refluxed (80 °C) for 16 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in EtOAc and washed with sodium thiosulfate (0.1 N) and water. The organic phase was dried over magnesium sulfate, filtered, and evaporated to dryness. The iodo derivative **6** was purified by preparative HPLC (MeOH/H_2O 90:10, t_R = 44 min) and crystallized from ether as a white powder (24 mg, 75%): mp 65–66 °C; TLC, R_f 0.53; UV (EtOH) 281, 287 min; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 0.99–2.35 (br m, 32 H, chain and steroid), 2.67–2.91 (m, 2 H, 6-CH_2), 3.18 (t, 2 H, J = 7.0 Hz, CH_2I), 3.75 (t, 1 H, J = 8.2 Hz, 17-H), 4.62 (s, 1 H, ArOH), 6.55 (d, 1 H, J = 2.8 Hz, 4-H), 6.63 (dd, 1 H, J = 8.4, 2.9 Hz, 2-H), 7.15 (d, 1 H, J = 8.5 Hz, 1-H); MS m/z 552 (27, M^+), 424 (1), 271 (1), 253 (15), 171 (22), 157 (100), 145 (47). Anal. (HRMS) Calcd for $C_{29}H_{45}O_2I$ 552.2466, found 552.2462. Anal. C, H. I.

7α-(11-Phenoxyundecyl)estra-1,3,5(10)-triene-3,17β-diol (7). A mixture of the bromo derivative **4** (54 mg, 0.11 mmol), phenol (20 mg, 0.21 mmol), and sodium bicarbonate (40 mg, 0.48 mmol) in DMF (10 mL) was stirred at 90 °C for 15 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in EtOAc and washed with HCl (10%) and a saturated NaCl solution. The organic layer was dried over magnesium sulfate, filtered, and evaporated to dryness. The compound **7** was purified by preparative HPLC (MeOH/H_2O 93:7, t_R = 28 min) and crystallized from ether as a white powder (8.6 mg, 15%): mp 52–53 °C; TLC, R_f 0.56; UV (EtOH) 279, 272, 265, 287 nm; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 1.15–2.30 (br m, 32 H, chain and steroid), 2.67–2.91 (m, 2 H, 6-CH_2), 3.75 (t, 1 H, J = 8.3 Hz, 17-H), 3.95 (t, 2 H, J = 6.6 Hz, CH_2OAr), 6.54 (d, 1 H, J = 2.8 Hz, 4-H), 6.62 (dd, 1 H, J = 8.4, 2.7 Hz, 2-H), 6.88–6.96 (m, 3 H, ArH ortho and para to CH_2O), 7.15 (d, 1 H, J = 8.4 Hz, 1-H), 7.24–7.31 (m, 2 H, ArH meta to CH_2O); MS m/z 518 (16, M^+), 500 (7), 424 (1), 271 (7), 253 (32), 171 (22), 157 (100), 145 (36), 94 (78, phenol$^+$). Anal. (HRMS) Calcd for $C_{35}H_{50}O_3$ 518.3760, found 518.3755. Anal. C, H.

7α-[11-(4-Iodophenoxy)undecyl]estra-1,3,5(10)-triene-3,17β-diol (8). A mixture of **4** (34 mg, 0.067 mmol), *p*-iodophenol (29 mg, 0.13 mmol), and sodium bicarbonate (25 mg, 0.30 mmol) in DMF (6.3 mL) was stirred at 90 °C for 15 h. After evaporation of the solvent under reduced pressure, the residue dissolved in EtOAc was washed with HCl (10%) and a saturated NaCl solution. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness. The compound **8** was purified by preparative HPLC (MeOH/H_2O 93:7, t_R = 40 min) and crystallized from ether as a white powder (19 mg, 45%): mp 66–67 °C; TLC, R_f 0.59; UV (EtOH) 281, 287, 273 nm; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 0.99–2.34 (br m, 32 H, chain and steroid), 2.67–2.91 (m, 2 H, 6-CH_2), 3.75 (t, 1 H, J = 8.3 Hz, 17-H), 3.90 (t, 2 H, J = 6.6 Hz, CH_2OArI), 4.67 (s, 1 H, ArOH), 6.54 (d, 1 H, J = 2.8 Hz, 4-H), 6.62 (dd, 1 H, J = 8.4, 2.7 Hz, 2-H), 6.65–6.70 (dm, 2 H, J = 9 Hz, ArH ortho to CH_2O), 7.15 (d, 1 H, J = 8.4 Hz, 1-H), 7.50–7.56 (dm, 2 H, J = 9 Hz, ArH meta to CH_2O); MS m/z 644 (13, M^+), 626 (1), 424 (2), 271 (7), 253 (18), 220 (70, *p*-iodophenol$^+$), 171 (30), 157 (100), 145 (54). Anal. (HRMS) Calcd for $C_{35}H_{49}O_3I$ 644.2728, found 644.2718. Anal. C, H. I.

7α-(10-Undecenyl)estra-1,3,5(10)-triene-3,17β-diol (9). The elimination product **9** was produced as a minor product in the nucleophilic substitution reactions during the synthesis of **3–8**. Compound **9** was purified by preparative HPLC (MeOH/H_2O 94:6, t_R = 39 min) and crystallized by evaporation of the solvent under reduced pressure as a white powder: mp 203–204 °C; TLC,

R_f 0.48; UV (EtOH) 280, 287, 273 nm; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3 H, 18-CH_3), 0.91–2.36 (br m, 31 H, chain and steroid), 2.70–2.92 (m, 2 H, 6-CH_2), 3.74 (t, 1 H, J = 8.3 Hz, 17-H), 4.05–4.23 (m, 3 H, CH=CH_2), 6.63 (d, 1 H, J = 2.7 Hz, 4-H), 6.73 (dd, 1 H, J = 8.5, 2.7 Hz, 2-H), 7.18 (d, 1 H, J = 8.6 Hz, 1-H); MS m/z 424 (100, M^+), 271 (1), 253 (2), 171 (9), 157 (26), 145 (16). Anal. (HRMS) Calcd for $C_{29}H_{44}O_2$ 424.3341, found 424.3348. Anal. C, H.

Estrogen Receptor Binding Assay. The relative binding affinity (RBA) of the estradiol derivatives for estrogen receptors was determined by the displacement of 17β-[^{3}H]estradiol. Competitive binding assays were done as previously described,[7,13,15] with some minor modifications. Calf uterine cytosol was prepared by centrifugation (105000g, 60 min, 4 °C) of homogenates in phosphate buffer (5 mM sodium phosphate, pH 7.5, 10 mM monotioglycerol, 10% glycerol) and diluted with phosphate buffer to 0.85 mg of protein/mL (0.68 mg of protein in the final incubation mixture). Cytosols were incubated with a constant concentration (10^{-9} M) of 17β-[^{3}H]estradiol (Amersham, 140 Ci/mmol) and nine different concentrations (3×10^{-12}–10^{-6} M) of test compounds at 4 °C for 2, 12, and 20 h in the absence of DMF, and 20 h with the addition of 7% DMF. Each concentration was performed in triplicate. Incubations were stopped by adding 500 μL of a dextran-coated-charcoal slurry (0.25% charcoal Norit A, 0.025% dextran, in 0.01 M tris(hydroxymethyl)aminomethane (pH 8.0), and the mixture was agitated at 4 °C for 30 min. After centrifugation, the radioactivity of a 500-μL supernatant aliquot was counted. The RBA value of a competitor was established by using the ratio of unlabeled 17β-estradiol concentration required for 50% receptor displacement of the corresponding 17β-[^{3}H]estradiol and the competitor concentration required for the same effect, multiplied by 100. The RBA reported are the average values of at least three experiments.

Acknowledgment. Financial support for this work was provided by the Medical Research Council of Canada.

Registry No. 1, 434-22-0; 2, 55592-09-1; 3, 123266-46-6; 4, 105089-28-9; 5, 123266-47-7; 6, 105075-33-0; 7, 123266-48-8; 8, 123266-49-9; 9, 123266-50-2; phenol, 108-95-2; p-iodophenol, 540-38-5.

Antifolate and Antibacterial Activities of 5-Substituted 2,4-Diaminoquinazolines

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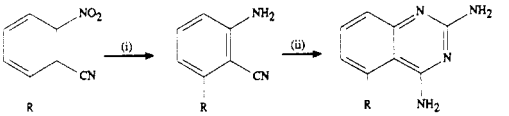
A series of 5-substituted 2,4-diaminoquinazolines (**3**) has been synthesized and evaluated as inhibitors of the enzyme dihydrofolate reductase (DHFR) from both bacterial and mammalian sources. The best compounds (e.g. **53**) show good activity against *Escherichia coli* DHFR, but there is no significant selectivity for the bacterial over the mammalian enzyme. The structure–activity relationships for enzyme inhibition appear to be complex and not amenable to simple analysis; a hypothesis to explain the observed qualitative structure–activity relationships is proposed. The inhibitory activities of the compounds against the growth of intact bacterial cells in vitro closely parallel those for the inhibition of the isolated bacterial enzymes, suggesting that their antifolate action is responsible for their antibacterial effects. Five of the compounds were tested for their ability to cure a systemic *E. coli* infection in the mouse, but they showed no therapeutic effects at their maximum tolerated doses.

Inhibitors of the enzyme dihydrofolate reductase (DHFR; tetrahydrofolate:NADP$^+$ oxidoreductase, EC 1.5.1.3) constitute an important class of therapeutic agents which have found application in anticancer (e.g. methotrexate),[1] antibacterial (e.g. trimethoprim),[2] and antimalarial (e.g. pyrimethamine)[3] chemotherapy. Since the discovery of the mode of action of methotrexate, DHFR inhibitors have been studied intensively and exhaustively. Interest in the development of new molecules of this class remains high and has been further stimulated by the recent publication of the X-ray crystallographic coordinates of various enzyme–inhibitor complexes.[4] Effective inhibitors of DHFR are limited to those compounds having the 2,4-diamino-1,3-diaza pharmacophore,[5] as exemplified by the 2,4-diaminopteridines (especially as anticancer agents), the 2,4-diaminopyrimidines (antibacterial, anticancer, and antimalarial), and the 2,4-diaminotriazines (anticancer and antimalarial). There are reviews available on all aspects of DHFR and its inhibitors.[5,6]

2,4-Diaminoquinazoline-based inhibitors of DHFR have also shown some antifolate properties that might make them useful in the treatment of malaria[7] and some cancers.[8] However, despite some promising early reports in the literature,[9] little attention has been paid to their antibacterial properties. Therefore, we decided to assess their potential as antibacterial agents. In order to do this a number of 2,4-diaminoquinazolines bearing carefully se-

Scheme I[a]



1 2 3

[a] (i) reduction, (ii) chloroformamidine hydrochloride/diglyme.

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