Synthesis and Biological Evaluation of 17β-Alkoxyestra-1,3,5(10)-trienes as Potential Neuroprotectants Against Oxidative Stress

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17β-*O*-Alkyl ethers (methyl, ethyl, propyl, butyl, hexyl, and octyl) of estradiol were obtained from 3-*O*-benzyl-17β-estradiol with sodium hydride/alkyl halide, followed by the removal of the *O*-benzyl protecting group via catalytic transfer hydrogenation. An increase compared to estradiol in the protection of neural (HT-22) cells against oxidative stress due to exposure of glutamate was furnished by higher (C-3 to C-8) alkyl ethers, while methyl and ethyl ethers decreased the neuroprotective effect significantly. Lipophilic (butyl and octyl) ethers blocking the phenolic hydroxyl (3-OH) of A-ring were inactive.

Introduction

Estrogens have long been recognized as antioxidants in a variety of in vitro and in vivo models. The antioxidant action is believed to be due to their ability to scavenge free radicals that cause neuronal cell death. Oxidative stress has been linked to neuronal cell death resulting from either acute insults due to ischemia, trauma, or chronic neurodegenerative diseases such as Alzheimer's disease (AD)[2] characterized by a progressive loss of memory and cognitive function. Although the histopathogenesis of AD is yet to be fully understood, one theory hypothesizes that the oxidative microenvironment surrounding the accumulated amyloid-β-peptide (Aβ) plaques is responsible for peroxidation of cell membrane lipids leading to cell lysis and death.[3] Another oxidative stressor suggested in AD's pathogenesis is the amino acid glutamate, the major excitatory neurotransmitter in the central nervous system.[4]

Population studies have shown that estrogen replacement therapy in postmenopausal women can decrease the incidence of AD or delay its onset.[4] It has been demonstrated[5] that the most biologically active estrogen, 17β-estradiol (**1**), is a potent antioxidant and has neuroprotective activity; however, the mechanism of action is still unclear. The direct neuroprotective effects of **1** on SK-N-SH human neuroblastoma cells under serum deprivation were first reported in 1994.[6] Numerous recent studies have demonstrated similar effects of **1** against a variety of toxicities, including oxidative stress,[7] in different types of neuronal cells. Interestingly, an enantiomer of estradiol ("*ent*-estradiol"), its epimer (17α-estradiol), and estratrien-3-ol are equipotent to **1** in protection of neural cells against oxidative damage, although they have significantly reduced estrogenic

activities.[6,8] All neuroprotective derivatives or analogues of **1** possess, however, a common structural element which is an intact, unsubstituted phenolic A-ring with its free 3-hydroxyl group.[9] Since lipophilic phenols also protect neural cells against glutamate and peroxide-mediated oxidative damage and cell death,[10] we decided to functionalize the 17-hydroxyl function of **1** by preparing alkyl ethers having a wide range of lipophilicity. We hypothesized that these ethers not only retain but also eventually increase neuroprotection compared to **1**. (In the meantime, these 17β-*O*-ethers also are expected to have a significantly less "feminizing" effect than **1**.) As controls, lipophilic 3-*O*-alkyl ethers have also been prepared and evaluated to further confirm the structural requirements of a phenolic A-ring for neuroprotection.

Chemistry

The previously reported preparation of 17β-methylestradiol[11] could have offered an attractive and convenient entry to a series of 17β-alkylestradiols. Unfortunately, we were unable to reproduce the procedure. Since alkylation on the phenolic 3-hydroxyl group proceeds under much milder conditions than that at the 17-position, we decided to protect the 3-OH selectively (and reversibly) before alkylating on the 17-position under strong basic conditions with the relevant alkyl halide. However, the initially prepared 3-*tert*-butyldimethylsilylated estradiol proved to be too unstable in both very mild acidic or basic conditions against alkyl halides, which resulted in a rapid desilylation followed by alkylation of the 3-OH. Similarly, the commercially available 3-benzoylestradiol was also unsuitable for the preparation of 17-alkylated ethers, since the phenolic ester group also hydrolyzed rapidly under the condition of our attempted alkylations. Therefore, the protection of the 3-OH of **1** as benzyl (Bz) ether[12] (**2**) was employed, followed by elaboration of the 17β-OH to the corresponding 17β-alkoxyl congeners **3a**−**f**. The 17-OH group was successfully alkylated with the corresponding alkyl halide in the presence of sodium hydride in DMF. However, the subsequent removal of the 3-benzyl pro-

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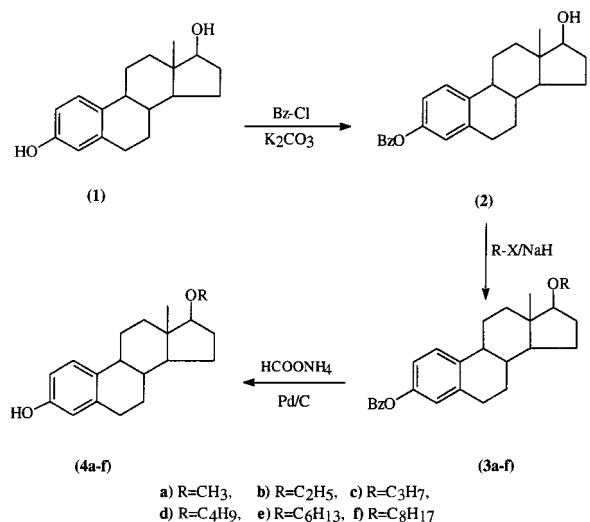† Center for Drug Discovery.
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Scheme 1. Synthesis of 17- and 3-Alkyl Ethers of Estradiol

a) R=CH$_3$, b) R=C$_2$H$_5$, c) R=C$_3$H$_7$,
d) R=C$_4$H$_9$, e) R=C$_6$H$_{13}$, f) R=C$_8$H$_{17}$


b) R'= C$_4$H$_9$, c) R'=C$_8$H$_{17}$

Figure 1. ORTEP plot of the X-ray crystal structure of 17-*O*-butylated 17β-estradiol **4d**. Thermal ellipsoids are shown at the 30% probability level.

tecting group was extremely slow in the usual fashion by using a Parr hydrogenator with Pd/C as the catalyst in glacial acetic acid. On the other hand, the 3-Bz protecting group was removed rapidly under ambient conditions by catalytic transfer hydrogenation using ammonium formate resulting in the desired products **4a**−**f**.[13,14] 3-*O*-Butyl and -octyl ethers of **1** (**5b**,**c**; Scheme 1) as controls were prepared directly from **1** by using alkyl halide in the presence of potassium carbonate.

In addition to NMR, mass spectrometry, chromatographic, and combustion analyses to characterize the compounds prepared, crystallography data were obtained for two representative 17β-ethers (**4a**,**d**). The solid-state conformation (ORTEP-type plot) of **4d** is shown in Figure 1. The crystals were monoclinic and belong to the *P*2(1) space group, and this confirmed that the 17-methoxy and -butoxy groups assumed a β-orientation in the D-ring.

Results and Discussion

Cytotoxicity studies on the compounds involved were done on mouse clonal hippocampal HT-22 cells, and the Calcein AM assay was used to quantify cell viability because it is robust, reproducible, has high capacity, and reliably determines cell viability against a variety of insults.[15] We have previously found this assay to be the most reliable and reproducible of all of the viability assays used. [For example, in an exhaustive assessment of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) versus Calcein AM in measuring toxicity induced by Aβ, we observed that Calcien AM, but not MTT, accurately measured viability − an observation similar to that reported by others;[16] both assays were equally effective in detecting H$_2$O$_2$-induced toxicity on the other hand.[17]]
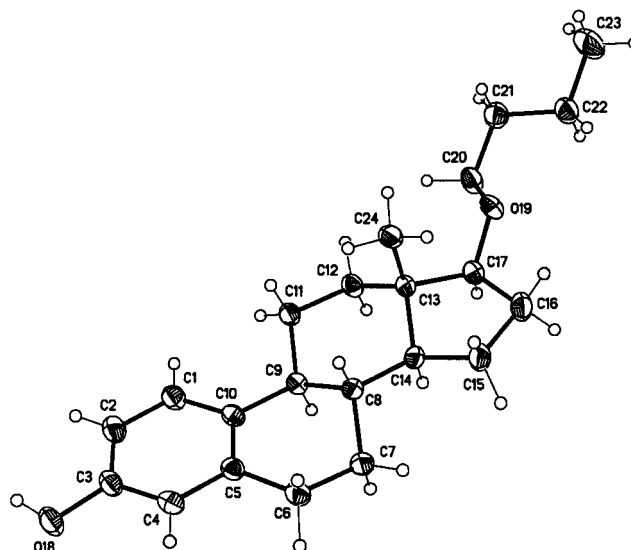
When testing the neuroprotective potency of estrogens, the ED$_{50}$ for estrogen neuroprotection is in the nanomolar range by dye exclusion techniques,[17] morphological criteria/cell counting,[18] and vital dyes (Calcein AM),[19] whereas ED$_{50}$s in the micromolar range were observed when MTT reduction is used as an endpoint. We have abandoned the frequently used trypan blue exclusion assay, as it requires the application of an additional stress − the harvesting of cells to conduct counts. This additional stress confounds the data. On the other hands, lactic acid dehydrogenase (LDH) release from dead or dying cells was highly variable in our hands and was too cumbersome to be used for screening in this study.

Compared to **1**, only **4c**−**f** of the six 17β-*O*-alkylestradiols tested improved neuroprotection in a dose-dependent manner against the glutamate-induced oxidative damage in murine HT-22 cells at concentrations of 0.1 μM and higher (Figure 2). These compounds were essentially equipotent at 1 μM (approximately twice as many cells were viable compared to the control) and showed no apparent relationship with a single molecular property such as lipophilicity (based on the calculated log P[20]) or water solubility (according to our solubility studies all of the tested compounds are essentially water-insoluble). The butyl (**4d**) and octyl (**4f**) ethers were, for example, no more neuroprotective at a concentration of 10 μM than at 1 μM. The parent compound (**1**) was effective only at 10 μM but was clearly superseded by **4c**,**e** at this concentration. 17β-Methylestradiol (**4a**) was also ineffective below 10 μM and decreased cell viability compared to **1** at 10 μM, while 17β-ethylestradiol (**4b**) were ineffective even at 10 μM. Similarly to earlier observations regarding the inability of 3-*O*-methylestradiol to exert neuroprotection,[11] **5b**,**c** ethers blocking the phenolic hydroxyl in the A-ring also were inactive.

The complex relationship of neuroprotection and 17-alkoxy chain length was surprising. A comparison of the solid-state conformation of **4a**,**d** revealed no apparent differences in the preferred geometry of the steroid backbone between a representative "active" (**4d**) and an
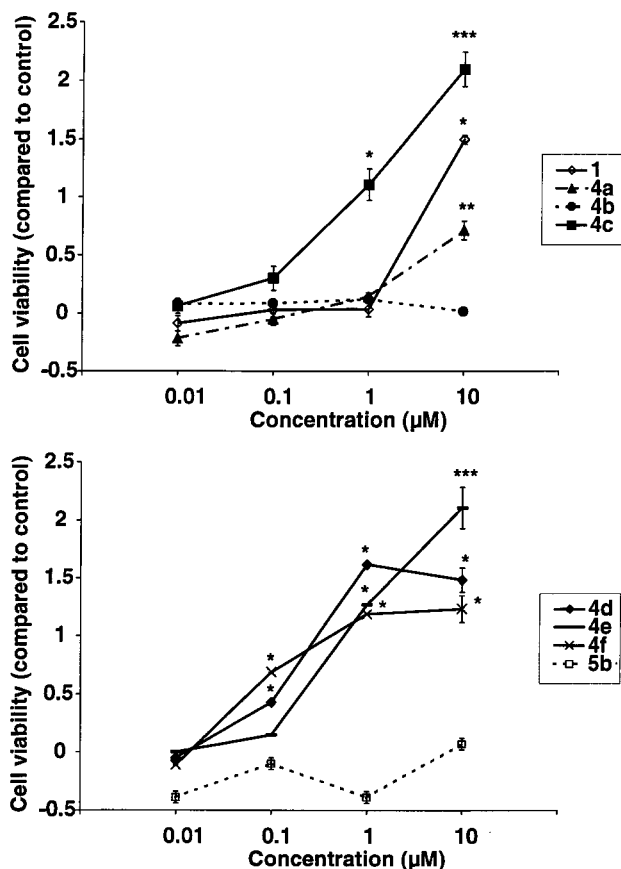
Figure 2. HT-22 cell viability in vitro after glutamate exposure (20 mM) following treatment with estradiol (**1**), its 17β-alkyl ethers (**4a−f**), and 3-butylestradiol (**5b**, as a typical representative of the 3-alkyl ethers). Statistically significant differences between groups were tested by analysis of variance (ANOVA) followed by post hoc Tukey test: *significant increase ($p < 0.05$) vs vehicle control, **significant increase ($p < 0.05$) vs vehicle control but decrease compared to 10 μM estradiol (**1**), ***significant increase ($p < 0.05$) vs vehicle control and statistically significant increase compared to 10 μM estradiol (**1**).

"inactive" (**4a**) ether derivative of **1**. One possible explanation is that the interaction of the alkyl chain of the 17β-substituent with the target site or the lipoidal cell membrane plays an important role in the efficacy of the derivative as a neuroprotectant. Thus, **4a**,**b** having a compact alkyl group may not have the flexibility (i.e., sufficient degrees of freedom for bond rotation) to embed into a cell membrane effectively; however, a longer alkyl chain (C $\geq$ 3) may provide this property. We also noted that the two "inactive" 17-ethers (**4a**,**b**) were compounds with high melting points, while the active ones have significantly lower melting points. Further studies correlating neuroprotection by phenolic A-ring steroids (including **4a−f**) with their effect on membrane fluidity are underway.

In summary, our results indicate that higher 17β-alkyl ethers of estradiol (**4c−f**) show a dose-dependent neuroprotection in vitro against oxidative stress in HT-22 cells. Moreover, this effect is manifested at lower concentration (<1 μM) than that of the parent compound (**1**).

Experimental Section

Instruments and Materials. All solvents and materials were obtained from Fisher Scientific (Atlanta, GA) or Aldrich (Milwaukee, WI). Estradiol (**1**) and 3-*O*-methyl-17β-estradiol (**5a**) were purchased from Sigma (St. Louis, MO). Sodium hydride was used as a 60% dispersion in mineral oil. Melting points were determined on a Fisher-Johns melting point apparatus and uncorrected. Thin-layer chromatography (TLC) was done on Whatman silica gel plates (on aluminum backing) containing UV fluorescence indicator by using ethyl acetate: hexane (1:4, v/v) eluent. All chromatographic purifications were done on gravity columns with 230−435 mesh neutral silica gel with ethyl acetate:hexane (1:4, v/v) as an eluent. Elemental analyses were performed by the Atlantic Microlab, Inc. (Norcross, GA). NMR spectral data were recorded for all compounds using a Varian XL-300 spectrometer and TMS as an internal standard. Mass spectral data were obtained by using atmospheric-pressure chemical ionization (APCI) on a quadrupole ion trap instrument (LCQ, Finnigan MAT, San Jose, CA). Analytical reversed-phase high-performance liquid chromatography was performed on a ThermoSeparation/SpectraPhysics (Fremont, CA) system consisting of an SP8810 isocratic pump, a Rheodyne (Cotati, CA) model 7125 injector valve equipped with a 20-μL sample loop, an SP8450 variable wavelength UV/VIS detector operated at 280 nm, and an SP4290 computing integrator. A 15-cm × 4.6-mm i.d. octade-cylsilica column (Phase Sep S5 ODS2, Queensferry, Clwyd, U.K.) and a mobile phase of acetonitrile containing 1% acetic acid at a flow rate of 1.0 mL/min were used for the analyses.

X-ray crystallography data were collected at 173 K on a Siemens SMART PLATFORM equipped with A CCD area detector and a graphite monochromator utilizing Mo Kα radiation ($\lambda = 0.71073$ Å). Cell parameters for each structure were refined using up to 8192 reflections and a hemisphere of data (1381 frames) was collected using the ω-scan method (0.3° frame width). The first 50 frames were remeasured at the end of data collection to monitor instrument and crystal stability (maximum correction on I was <1%). Absorption corrections by integration were applied based on measured indexed crystal faces. Both structures were solved by the Direct Methods in SHELXTL5[21] and refined using full-matrix least-squares. The non-H atoms were treated anisotropically, whereas the hydrogen atoms were calculated in ideal positions and were riding on their respective carbon atoms, except the hydroxyl protons H$_{18}$ in **4a** and H$_{26}$ in **4d**. These protons were obtained from a difference Fourier map and refined without any constraints. While no solvent crystallized with **4a**, a methanol molecule was found in the general position in the lattice of **4d**. A total of 196 parameters of **4a** were refined in the final cycle of refinement using 2961 reflections with $I >$ 2σ(I) to yield R_1 and wR_2 of 5.03% and 12.66%, respectively. For **4d**, a total of 247 parameters were refined in the final cycle of refinement using 3294 reflections with $I > 2\sigma(I)$ to yield R_1 and wR_2 of 3.71% and 8.90%, respectively. Refinement was done using F^2. Tables of geometric data, indicating H-bonding interactions, are available as Supporting Information.

3-Benzyloxyestra-1,3,5(10)-trien-17β-ol (2).[12] To 5 g (18 mmol) of **1** and 10 g (72 mmol) of potassium carbonate in 100 mL of dry acetone was added 5.7 g (4.0 mL, 34 mmol) of benzyl bromide. The mixture was refluxed overnight under nitrogen atmosphere. Upon cooling the solid was removed by filtration. The filtrate was collected and acetone was removed in vacuo leaving behind clear yellowish oil, which solidified on standing. Recrystallization from ethyl acetate/hexane gave 6.1 g (93% yield) of a white fluffy solid: mp 61−63 °C; TLC R_f 0.23; ^{1}H NMR (CDCl$_3$) δ 7.44−7.19 (m, 5H, C$_6$H$_5$ of benzyl), 7.12 (d, $J = 8.6$ Hz, 1H, 1-CH), 6.78 (dd, $J = 8.7$ and 2.7 Hz, 1H, 2-CH), 6.72 (d, $J = 2.4$ Hz, 1H, 4-CH), 5.05 (s, 3H, OCH$_2$ of benzyl), 3.37 (tr, $J = 8.4$ Hz, 1H, 17α-CH), 2.87−2.82 (m, 2H, 6-CH$_2$), 2.34−1.18 (m, 13H), 0.78 (s, 3H, 13-CH$_3$); MS m/z 363 [M + H]$^+$.

General Procedure for Preparation of 3-Benzyloxy-17β-alkoxyestra-1,3,5(10)-trienes 3a−f. Compound **2** (0.8 g, 2.2 mmol) was dissolved in 5 mL of anhydrous DMF and, then, sodium hydride (0.3 g) was added. The mixture was stirred at room temperature for 30 min before the addition of 20 mmol

of alkyl halide. The stirring was continued overnight. The reaction mixture was quenched by pouring it into 20 mL of diluted hydrochloric acid and extracted with methylene chloride. The organic phase was dried over Na_2SO_4 and the solvent removed in vacuo leaving behind a clear, yellowish oil which solidified on standing. The crude products were purified by either recrystallization or column chromatography. Synthesis information and analytical data of a representative compound (**3a**) in the series are given below; see Supporting Information for those of **3b**−**f**.

3-Benzyloxy-17β-methoxyestra-1,3,5(10)-triene (3a): recrystallization from methanol, 63% yield, yellowish solid; mp 92−94 °C; TLC R_f 0.83; ^{1}H NMR (CDCl$_3$) δ 7.48−7.32 (m, 5H, C$_6$H$_5$ of benzyl), 7.27 (d, J = 8.7 Hz, 1H, 1-CH), 6.81 (dd, J = 8.7 and 2.1 Hz, 1H, 2-CH), 6.73 (d, J = 2.4 Hz, 1H, 4-CH), 5.05 (s, 2H, OCH$_2$ of benzyl)), 3.39 (s, 3H, 17β-OCH$_3$), 3.33 (t, 1H, J = 8.7 Hz, 17α-CH), 2.83 (m, 2H, 6-CH$_2$), 1.22−2.34 (m, 13H), 0.80 (s, 3H, 13-CH$_3$); MS m/z 377 [M + H]$^+$.

General Procedure for Preparation of 17β-Alkoxy-estra-1,3,5(10)-trienes 4a−f. To a solution of 2.0 mmol of **3a**−**f** in 10 mL of methanol were added 0.2 g of Pd/C (10%) and ammonium formate (1.00 g, 16 mmol). The reaction mixture was stirred at room temperature for 1 h. Pd/C was then removed by filtration and solvent was evaporated in vacuo. To the oily residue water was added and the resulting solid was collected by filtration. Either recrystallization or column chromatography was used for purification. Synthesis information and analytical data of a representative compound (**4a**) in the series are given below; see Supporting Information for those of **4b**−**f**.

17β-Methoxyestra-1,3,5(10)-trien-3-ol (4a): recrystallization from methanol, 50% yield, white solid; mp 242−244 °C; TLC R_f 0.48; ^{1}H NMR (DMSO) δ 7.05 (d, J = 8.40 Hz, 1H, 1-CH), 6.51 (dd, J = 8.40 and 2.10 Hz, 1H, 2-CH), 6.45 (d, J = 2.40 Hz, 1H, 4-CH), 3.30 (s, 3H, 17β-OCH$_3$), 3.28 (t, J = 8.25 Hz, 1H, 17α-CH), 2.73−2.72 (m, 2H, 6-CH$_2$), 2.56−1.22 (m, 13H), 0.74 (s, 3H, 13-CH$_3$); ^{13}C NMR (DMSO) δ 156.7(C-3) 139.3 (C-5 or C-10), 132.7 (C-10 or C-5), 128.0 (C-1), 116.8 (C-2 or C-4), 114.5 (C-4 or C-2), 92.2, 58.7 (OCH$_3$), 51.7 (C-17), 45.6, 44.6, 40.2, 39.8, 31.1, 29.2, 28.8, 28.1, 24.4, 13.6 (13-CH$_3$); MS m/z 287 [M + H]$^+$, 255 [M − OCH$_3$]$^+$. Anal. C, H.

General Procedure for Preparation of 3-Alkoxyestra-1,3,5(10)-trienes 5b,c. To a suspension of compound **1** (0.5 g, 1.8 mmol) and potassium carbonate (1.00 g, 7.2 mmol) in 5 mL of dry acetone was added 10 mmol of 1-bromoalkane. The mixture was refluxed overnight then allowed to cool and it was filtered. The acetone was removed in vacuo and the oily residue was purified. Synthesis information and analytical data of a representative compound (**5b**) in the series are given below; see Supporting Information for those of **5c**.

3-Butoxyestra-1,3,5(10)-trien-17β-ol (5b): recrystallization from methanol:water (1:1, v/v), 68% yield, white solid; mp 86−88 °C; TLC R_f 0.62; ^{1}H NMR (CDCl$_3$) δ 7.17 (d, J = 8.7 Hz, 1H, 1-CH), 6.70 (dd, J = 8.4 and 2.40 Hz, 1H, 2-CH), 6.62 (d, J = 2.4 Hz, 1H, 4-CH), 3.93 (t, J = 6.30 Hz, 2H, OCH$_2$ of 3-OC$_4$H$_9$), 3.71 (t, J = 8.1 Hz, 1H, 17α-CH), 2.86−2.80 (m, 2H, 6-CH$_2$), 2.20−1.10 (m, 17H, including (CH$_2$)$_2$ of 3-OC$_4$H$_9$), 0.96 (t, J = 7.4 Hz, 3H, CH$_3$ of 3-OC$_4$H$_9$); ^{13}C NMR (CHCl$_3$) δ 156.9 (C-3), 137.7 (C-5 or C-10), 132.3 (C-10 or C-5), 126.1 (C-1), 114.4, 111.9, 81.7, 67.5 (OCH$_2$ of 3-OC$_4$H$_9$), 49.9, 43.8, 43.1, 38.7, 36.6, 31.3, 30.4, 29.7, 27.2, 26.3, 23.0, 19.2, 13.7 (CH$_3$ of 3-OC$_4$H$_9$), 10.9 (13-CH$_3$); MS m/z 311 [M − OH]$^+$.

Cytotoxicity Studies. HT-22 cells (generously provided by Dr. David Schulbert, Salk Institute, La Jolla, CA) were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum under the usual conditions. All wells in the 96-well culture plate contained approximately 5000 cells as determined by a Neubauer hemacytometer and the cells were incubated for 24 h before the compounds were added. The estradiol derivatives purified by recrystallization or column chromatography were free from **1** as determined by HPLC. All agents were dissolved in absolute ethanol and diluted, with the culture media, to a final concentration of 0.01, 0.1, 1.0, and 10 μM in their respective wells. The cells were further incubated for 24 h before sodium glutamate in a solution of phosphate buffer was added. Cell viability was quantified 2 h later by the Calcein AM assay in a phosphate buffer solution.

Statistical Analysis. ANOVA was used to determine the significance of differences among groups. Comparison between groups were done using the Tukey test. A p < 0.05 was considered significant.

Supporting Information Available: Detailed information on the synthesis and characterization of **3b**−**f**, **4b**−**f**, and **5c**; X-ray crystallographic data of **4d**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Glenner, G. G. Alzheimer's disease: its proteins and genes. *Cell* **1988**, *52*, 307−308.

(2) Yankner, B. A. Mechanism of neuronal degeneration in Alzheimer's disease. *Neuron* **1996**, *16*, 921−936.

(3) Maher, P.; Davis, J. The role of monoamine metabolism in oxidative glutamate toxicity. *J. Neurosci.* **1996**, *15*, 6394−6401.

(4) (a) Paganni-Hill, A.; Henderson, V. Estrogen deficiency and risk of Alzheimer's disease in women. *Am. J. Epidemiol.* **1994**, *140*, 256−261. (b) Kawas, C.; Resnick, S.; Morrison, A.; Brookmeyer, R.; Corrasa, M.; Zonderman, A.; Basal, C.; Lingle, D. D.; Metter, E. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore longitudinal study of aging. *Neurology* **1997**, *48*, 1517−1521.

(5) Gridley, K. E.; Green, P. S.; Simpkins, J. W. A novel, synergistic interaction between 17β-estradiol and glutathione in the protection of neurons against β-amyloid 25−35-induced toxicity in vitro. *Mol. Pharmacol.* **1998**, *54*, 874−880.

(6) Bishop, J.; Simpkins, J. W. Estradiol treatment increases viability of glioma and neuroblastoma cells in vitro. *Mol. Cell Neurosci.* **1994**, *5*, 303−308.

(7) (a) Behl, C.; Widmann, M.; Trapp, T.; Holspoer, F. 17β-estradiol protects neurons from oxidative stress-induced cell death in vitro. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 473−482. (b) Sawada, H.; Ibi, M.; Kihara, T.; Urushitani, M.; Akaike, A.; Shimohama, S. Estradiol protects mesencephalic dopaminergic neurons from oxidative stress-induced neuronal death. *J. Neurosci. Res.* **1998**, *54*, 707−719.

(8) (a) Green, P. S.; Bishop, J.; Simpkins, J. W. 17α-estradiol exerts neuroprotective effects in SK-N-SH cells. *J. Neurosci.* **1997**, *17*, 511−515. (b) Behl, C.; Skutella, T.; Lezoualch, F.; Post, A.; Widmann, M.; Newton, C. J.; Holsber, F. Neuroprotection against oxidative stress by estrogens. Structure−activity relationship. *Mol. Pharmacol.* **1997**, *51*, 535−541.

(9) Green, P. S.; Gordon, K.; Simpkins, J. W. Phenolic A ring requirements for the neuroprotective effects of steroids. *J. Steroid Biochem. Mol. Biol.* **1997**, *63*, 229−235.

(10) Moorsmann, B.; Behl, C. The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic properties. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8867−8872.

(11) Coombs, M.; Roderick, H. 17β-O estradiol 17-methyl ether. *Steroids* **1965**, *6*, 841−4.

(12) Qian, X.; Abul-Hajj, Y. J. Synthesis and biological activity of 17β-substituted estradiol. *J. Steroid Biochem.* **1988**, *29*, 657−664.

(13) Anwer, M.; Spatola, A. An advantageous method for the rapid removal of hydrogenolysable protecting groups under ambient conditions; synthesis of leucine-enkephalin. *Synthesis* **1980**, 929−932.

(14) Elamin, B.; Anantharamaiah, G.; Royer, G.; Means, G. Removal of benzyl-type protecting groups from peptides by catalytic transfer hydrogenation with formic acid. *J. Org. Chem.* **1979**, *44*, 3442−3444.

(15) Green, P. S.; Perez, E. T.; Calloway, T.; Simpkins, J. W. Estradiol attenuation of β-amyloid-induced toxicity: A comparison of MTT and Calcein assays. *J. Neurocytol.*, in press.

(16) (a) Behl, C.; Davis, J. B.; Lesley, R.; Schubert, D. Hydrogen-peroxide mediates amyloid-beta protein toxicity. *Cell* **1994**, *77*, 817−827. (b) Shearman, M. S.; Ragan, C. I.; Iversen, L. L. Inhibition of PC12 cell redox activity is a specific, early indicator of the mechanism of beta-amyloid-mediated cell-death. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 470−1474.

(17) (a) Green P. S.; Gridley K. E.; Simpkins, J. W. Estradiol protects against β-amyloid (25−35)-induced toxicity in SK-N-SH human neuroblastoma cells. *Neurosci. Lett.* **1996**, *218*, 165−168. (b) Green P. S.; Gridley K. E.; Simpkins, J. W. Nuclear estrogen receptor-independent neuroprotection by estratrienes: A novel interaction with glutathione. *Neuroscience* **1998**, *84*, 7−10.

(18) Mook-Jung, I.; Joo, I.; Sohn, S.; Kwon, H. J.; Huh, K.; Jung, M. W. Estrogen blocks neourotoxic effect of β-amyloid (1−42) and induces neurite extension on B103 cells. *Neurosci. Lett.* **1997**, *235*, 101−104.

(19) Pike, J. Estrogen modulates neuronal Bcl-x$_l$ expression and β-amyloid-induced apoptosis. Relevance to Alzheimer's disease. *J. Neurochem.* **1999**, *72*, 1552−1563.

(20) The logarithm of the 1-octanol/water partition coefficient (log *P*) was calculated by an atom fragment method implemented in the molecular modeling package HyperChem version 6.0 (Hypercube, Gainesville, FL): Ghose, A. K.; Pritchett, A.; Crippen, G. M. Atomic physicochemical parameters for 3-dimensional structure directed quantitative structure−activity-relationships. 3. Modeling hydrophobic interactions. *J. Comput. Chem.* **1988**, *9*, 80−90. The obtained log *P* values were as follows: 4.01 (**1**), 4.29 (**4a**), 4.63 (**4b**), 5.10 (**4c**), 5.49 (**4d**), 6.29 (**4e**), and 7.08 (**4f**). The calculated log *P* values for the 3-alkylestradiols were 4.09 (**5a**), 5.25 (**5b**), and 6.83 (**5c**).

(21) Sheldrick, G. M. *SHELXTL5*; Bruker-AXS: Madison, WI, 1998.