

Tetrahydroisoquinolines as subtype selective estrogen agonists/antagonists

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Abstract—Two series of 6-hydroxy and 7-hydroxy tetrahydroisoquinolines were prepared. Evaluating a range of C-1, C-4, and N-substituents led to the discovery of ER α and ER β selective analogs.
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The major endogenous ligands that act at the estrogen receptors (ER) α and β are 17- β estradiol and estrone.¹ These two estrogen receptors are members of the nuclear hormone receptor superfamily,² which mediate a number of physiological processes such as bone mineral density and lipid levels as well as female reproductive systems.³ Post-menopausal women have severely decreased levels of endogenous estrogens and as such are often treated with HRT (hormone replacement therapy).⁴ HRT though, is not without risk. It is associated, for example, with an increased incidence of both breast and uterine cancers.⁵ Due to the side effects of HRT, the estrogen receptor(s) have been a target of considerable interest for the pharmaceutical industry.⁶ Compounds targeted initially at ER α (at the time ER β was unknown),⁷ have been found to retain some of 17- β estradiol's positive effects, while overcoming some of the side effects. Molecules that have this mixed agonist/antagonist profile are referred to as SERMs (Selective Estrogen Receptor Modulators).⁸ Tamoxifen was the first clinically approved SERM, for the treatment of breast cancer.⁹ Tamoxifen however, is associated with an increased incidence of uterine cancer¹⁰ and second generation SERMs have managed to overcome this increased risk. Examples from this class include raloxifene¹¹ (clinically approved for the treatment of

osteoporosis) as well as lasofoxifene **1**,¹² currently in advanced clinical trials for osteoporosis. Since the discovery of these SERMs, major advances in the understanding of estrogens have occurred, not the least of which is the discovery of a second estrogen receptor, ER β .⁷ With the discovery of this second subtype it is hoped that compounds can be found with new pharmacology compared to the previously described SERMs. In our search for new agents, we chose to investigate the tetrahydroisoquinoline (THIQ) scaffold **2**. As can be seen from Figure 1, the THIQ scaffold is structurally similar to the tetrahydronaphthalene scaffold of lasofoxifene **1**, but incorporation of the nitrogen atom allows for the rapid synthesis of analogs. The first report of THIQs analogs as estrogenic compounds was made by Nagarajan et al.,¹³ who described their use as anti-implantation agents. More recently the Novartis group has

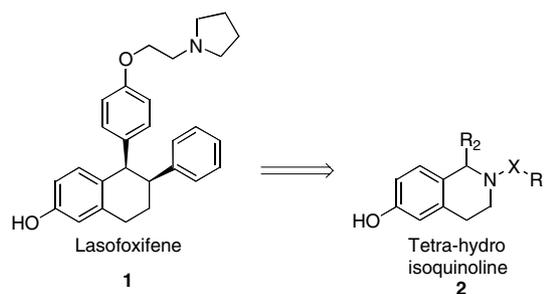
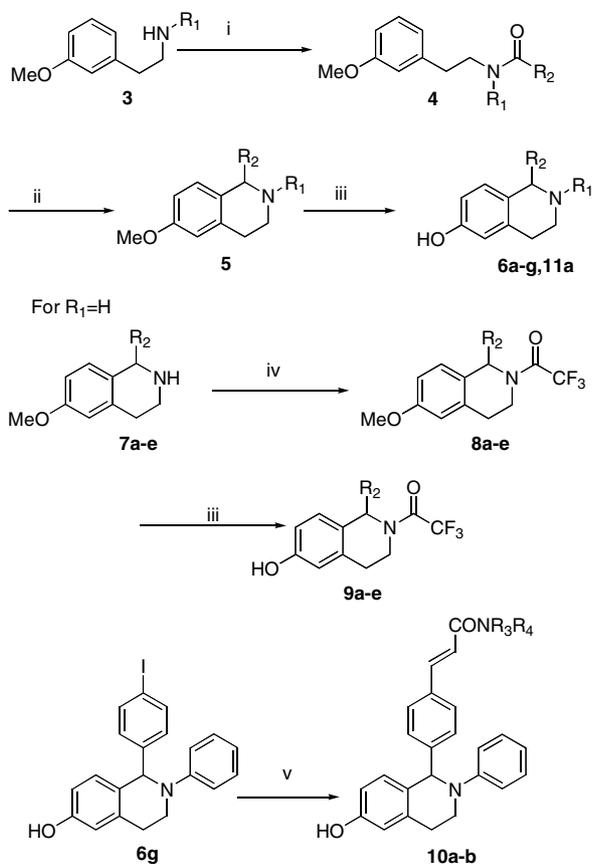


Figure 1. THIQs derived from lasofoxifene.

Keywords: Tetrahydroisoquinolines; ER α ; ER β .

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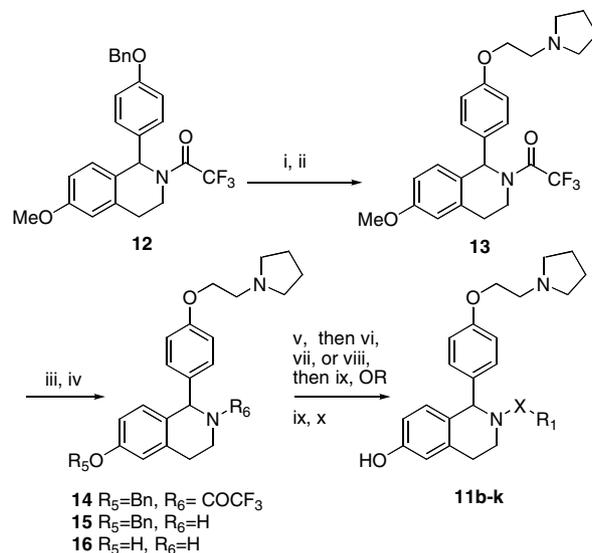


Scheme 1. Synthesis of compounds for **6a–g**, **9a–e**, **10a–b**, and **11a**. Reagents and conditions: (i) RCO_2H , Et_3N , CH_2Cl_2 1-propane phosphonic cyclic anhydride, DMAP or RCO_2H , Et_3N , CH_2Cl_2 , EDC, HOBT; (ii) POCl_3 , reflux or PCl_5 , 1,2 DCE reflux, then NaBH_4 , MeOH; (iii) BBr_3 , CH_2Cl_2 , -78°C to rt; (iv) TFAA, Et_3N , CH_2Cl_2 , 0°C ; (v) $\text{Pd}(\text{PPh}_3)_4$, Et_3N , $\text{H}_2\text{C}=\text{CHCONR}^3\text{R}^4$, DMF, 100°C .

released their results describing THIQs as possible SERMs.¹⁴

The THIQs were synthesized using standard chemical transformations, starting from the appropriate 3-methoxy phenethylamine **3** (Scheme 1). Treatment of 3-methoxy phenethylamine ($\text{R}_1 = \text{H}$ or Ph)¹³ with the appropriate R_2 containing carboxylic acid in the presence of the coupling agents such as 1-propane phosphonic acid cyclic anhydride (PPAA) for $\text{R}_1 = \text{Ph}$ and EDC/HOBt for $\text{R}_1 = \text{H}$ provided the desired amides **4**. The amide **4** underwent a Bischler–Napieralski cyclization in the presence of POCl_3 (CAUTION), that after reduction with NaBH_4 , gave the corresponding 6-methoxy tetrahydroisoquinoline **5**. When $\text{R}_1 = \text{phenyl}$, the methoxy protecting group was removed using BBr_3 to unmask the phenol giving the desired analogs **6a–f** and intermediate **6g**. The amines **7a–e** were capped with trifluoroacetic anhydride (TFAA) and the resultant intermediates **8a–e** were then subjected to BBr_3 to reveal the desired phenolic analogs **9a–e**. Examples **10a–b** were prepared from the THIQ **6g** via a Heck reaction with the appropriate acrylamide.

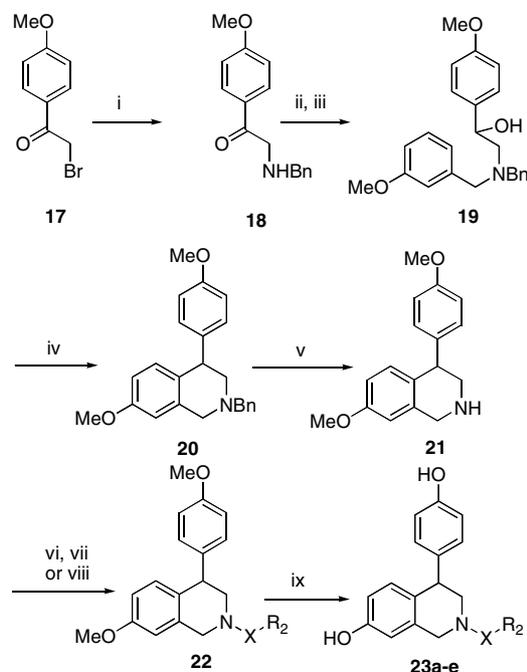
Compound **11a** was prepared using the chemistry depicted in Scheme 1 using 4-phenyl-(2-pyrrolidin-1-yl



Scheme 2. Synthesis of compounds **11b–k**. Reagents and conditions: (i) 10% Pd/C, H_2 44 psi, EtOH; (ii) NaH, DMF, 1-(2-chloroethyl) pyrrolidine HCl, 100°C ; (iii) BBr_3 , CH_2Cl_2 , 0°C ; (iv) NaH, BnBr, DMF, 100°C ; (v) K_2CO_3 , MeOH, reflux; (vi) RCOCl , Et_3N , THF; (vii) RSO_2Cl , Et_3N , THF; (viii) RCO_2Cl , Et_3N , THF; (ix) 20% $\text{Pd}(\text{OH})_2/\text{C}$, NH_4HCO_2 , MeOH, reflux; (x) RCHO , NaOAc, NaCNBH₃, MeOH.

ethoxy) benzoic acid. Compounds **11b–k** were synthesized as depicted in Scheme 2. Starting with the differentially protected THIQ **12** (synthesized as for **8a–e** in Scheme 1 using 4-benzyloxy carboxylic acid), the benzyl ether was selectively removed via hydrogenolysis and the resulting phenol was alkylated under basic conditions with 1-(2-chloroethyl) pyrrolidine hydrochloride to give **13**. The 6-methoxy group was removed and replaced as a benzyl ether to give **14** in order to facilitate analog synthesis. The trifluoroacetamide group in **14** was removed via basic hydrolysis with K_2CO_3 in MeOH to reveal the secondary amine **15**, which was capped with either an acid chloride, a sulfonyl chloride or a chloroformate to produce the corresponding amide, sulfonamide, or carbamate followed by transfer hydrogenolysis of the benzyl ether group to give the analogs **11b–i**. The basic amines **11j** and **11k** were synthesized from **15** via transfer hydrogenolysis followed by reductive amination using the appropriate aldehyde in the presence of NaCNBH_3 .

The 7-hydroxy THIQs analogs were synthesized as depicted in Scheme 3. The bromoketone **17** was converted into the amino-ketone **18** upon treatment with benzyl amine. The amino-ketone underwent reductive amination with 3-methoxy benzaldehyde, followed by reduction of the keto group with NaBH_4 , to give **19**. The amino-alcohol **19** underwent an acid mediated cyclization to give the 7-methoxy THIQ **20**, which upon hydrogenolysis of the N-benzyl group gave the intermediate **21**. The amide and sulfonamide analogs were prepared by treating the secondary amine **21** with the appropriate acid or sulfonyl chlorides or with TFAA to give **22**, which upon exposure to BBr_3 furnished the analogs **23a–d**. Compound **23e** was prepared directly from **20** by treatment with BBr_3 .



Scheme 3. General synthesis of 7-hydroxy THIQs. Reagents and conditions: (i) BnNH_2 , Et_3N , THF, rt; (ii) 3-methoxy benzaldehyde, $\text{NaB}(\text{OAc})_3\text{H}$, 1,2 DCE; (iii) NaBH_4 , MeOH, 0°C ; (iv) CH_2Cl_2 , TFA, reflux; (v) Pd/C, H_2 50 psi, EtOH; (vi) PhCOCl , Et_3N , CH_2Cl_2 ; (vii) RSO_2Cl , Et_3N , CH_2Cl_2 ; (viii) TFAA, Et_3N , CH_2Cl_2 , 0°C ; (ix) $\text{BBr}_3/\text{CH}_2\text{Cl}_2$, -78°C to rt.

We initially chose to keep $\text{R}_1 = \text{phenyl}$ analogous to lasofoxifene **1** and explore the space described by R_2 . We found that the space defined by R_2 was tolerant of a wide variety of substituents. As can be seen in Table 1, the introduction of just a simple phenyl group **6a** produces a reasonably potent analog. Interestingly, replacement of the phenyl group with a 2-thienyl group produced compound **6b**, which demonstrated modest selectivity (2 \times) for ER β . The addition of the dimethyl acrylamide **10a** or the morpholino acrylamide **10b** groups to the phenyl ring, improved potency at ER α by up to 10 \times . An aromatic group at R_2 was not necessary to retain potency, as evidenced by compounds **6c–e**. Introduction of a methylene spacer group at R_2 produced analogs **6e–f** with modest (up to 10 \times) selectivity for ER α . The R_1 phenyl group could be effectively replaced with a trifluoroacetamide group. The introduction of this group allowed us to access analogs **9a–e** that displayed modest selectivity for ER β . Compounds **9d** and **9e** were separated by chiral HPLC and as can be seen, the biological activity resides in one isomer only. Compound **9d** was putatively assigned as the R-enantiomer based on the stereochemistry of lasofoxifene **1**.

In Table 2 the results of our investigations into different R_1 groups can be seen where R_2 was kept constant as the phenyl alkoxyamine moiety found in lasofoxifene **1**. The direct THIQ analog **11a**[†] of lasofoxifene was found to be

[†] In Ref. 14 the selectivity for compound **11a** was found to be ca. 8 \times for ER α , whereas we found the selectivity to be ca. 2 \times . This difference in selectivity may be due to differing assay conditions.

Table 1. Estrogen receptor binding affinities (IC_{50}) for compounds **6–10**¹⁵

Compd	R_1	X	R_2	Binding affinity (nM) ^a	
				ER α	ER β
6a	Ph	—	Ph	30.7	48.8
6b	Ph	—	2-Thienyl	50.0	26.3
6c	Ph	—	Cyclohexyl	29.8	99.0
6d	Ph	—	<i>i</i> -Pr	42.7	180
6e	Ph	—	CH_2 -cyclohexyl	53.1	295
6f	Ph	—	CH_2Ph	6.70	66.4
9a	CF_3	CO	2-Thienyl	385	63.5
9b	CF_3	CO	Ph	235	27.4
9c	CF_3	CO	<i>p</i> - C_6H_4 -Br	165	22.0
9d ^b	CF_3	CO	<i>p</i> - C_6H_4 -OH	53.6	8.90
9e ^c	CF_3	CO	<i>p</i> - C_6H_4 -OH	>3200	>3200
10a	Ph	—	—	8.00	8.70
10b	Ph	—	—	5.90	4.80

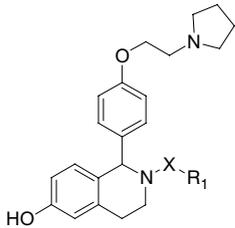
^a Values are means of at least three experiments.

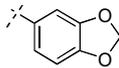
^b Single enantiomer. Tentatively assigned as the R-enantiomer.

^c Single enantiomer. Tentatively assigned as the S-enantiomer.

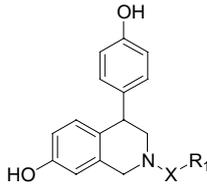
a good binder, but upon inserting an amide linker into the molecule **11b**, a considerable loss of potency was observed. Not all amides were impotent, as the trifluoroacetamide derivative **11c** exhibited improved potency. Interestingly, this analog showed a slight preference for ER α over ER β (ca. 3 \times) in contrast to analogs **9a–d**. This bias for ER α may result from the increased steric bulk of R_2 , which is consistent with ER β having a smaller ligand binding pocket than ER α .¹⁶ Upon replacing the amide linkage with a sulfonamide **11d** or a methylene linker **11j**, potency at both the receptors was restored. We also found that carbamate linkers were also well tolerated as seen with compound **11e**. We further investigated the scope of the sulfonamide linker and found that *para*-substitution of the phenyl sulfonamide led generally to less potent compounds **11f–h** than when compared to the parent sulfonamide **11d**. The 1-naphthyl sulfonamide **11i**, however, was almost as potent as the phenyl sulfonamide **11d** indicating that reasonable steric bulk could be tolerated if appropriately positioned. Interestingly, the two analogs that contain a methylene linker, **11j** and **11k**, both show ca. 10–20 \times selectivity for ER α over ER β .

The binding affinities of the 7-hydroxy THIQs **23a–e** are shown in Table 3. The 7-hydroxy THIQ scaffold is tolerant of the amide **23a**, sulfonamide **23b**, and methylene **23c** linkers. The potency of the amide **23a** is particularly

Table 2. Estrogen receptor binding affinities (IC₅₀) for compounds **1** and **11a–k**


Compd	X	R ₁	Binding affinity (nM) ^a	
			ER α	ER β
1	n/a	n/a	0.50	1.21
11a	—	Ph	11.5	22.2
11b	CO	Ph	562	2250
11c	CO	CF ₃	28.7	93.3
11d	SO ₂	Ph	5.20	18.5
11e	CO ₂	Et	44.3	293
11f	SO ₂	<i>p</i> -Tolyl	24.8	71.2
11g	SO ₂	<i>p</i> -C ₆ H ₄ - <i>n</i> Pr	29.4	133
11h	SO ₂	2-Naphthyl	112	1080
11i	SO ₂	1-Naphthyl	5.60	21.3
11j	CH ₂	Ph	4.70	42.2
11k	CH ₂		9.20	220

n/a: Not applicable.

^a Values are means of at least three experiments.**Table 3.** Binding affinities of 7-hydroxy THIQs


Compound	X	R ₁	Binding affinity (nM) ^a	
			ER α	ER β
23a	CO	Ph	160.0	27.9
23b	SO ₂	Ph	120.0	41.7
23c	SO ₂	1-naphthyl	28.0	10.7
23d	CO	CF ₃	122.7	11.4
23e	CH ₂	Ph	20.1	13.1

^a Values are means of at least three experiments.

interesting when contrasted to the 6-hydroxy THIQ benzamide **11b**. As with the 6-THIQs, the 1-naphthyl sulfonamide **23c** was also reasonably potent. The trifluoroacetamide analog **23d** proved to be the most ER β selective (ca. 10×) analog found during this investigation. In general we found the 7-hydroxy THIQ template to be selective for ER β over ER α.

A few select compounds were advanced into the MCF-7 cell proliferation assay as shown in Table 4. The MCF-7 cells are derived from breast tumors that predominantly express ER α.¹⁷ As anticipated, compounds that lack the phenyl alkoxy-amino sidechain at R₂, (e.g., compound

Table 4. MCF-7 cell proliferation data for selected compounds¹⁷

Compd	Agonist (nM)		Antagonist (nM)	
	EC ₅₀	% act. ^a	IC ₅₀	% inh. ^a
6c	11.1	93	n/a	n/a
11c	n/a	n/a	86.6	83
11f	n/a	n/a	29.8	66
11i	n/a	n/a	51.3	96

n/a: Not applicable.

^a Percent activation and inhibition are calculated from highest dose (1 μM).

6c) were found to be agonists.¹⁸ Compounds containing the phenyl alkoxy-amino sidechain at R₂, such as **11c**, **11f**, and **11i**, were found to antagonize the proliferative action of 17-β estradiol in this cell line thus confirming that this series could antagonize the estrogen receptor.

In summary, the THIQ scaffold has proved to be a versatile scaffold. It has allowed the identification of novel analogs that can act as ER agonists or antagonists in MCF-7 cells and also has led to the identification of ER α and ER β selective ligands. Through subtle changes of the R₁ and R₂ substituents, as well as the location of the phenolic group of the THIQ scaffold, compounds that are up to 20× selective for ER α, as well as compounds that are up to 10× selective for ER β have been identified.

References and notes

- Kuiper, G. G. J. M.; Carlsson, B.; Grandien, K.; Enmark, E.; Häggblad, J.; Nilsson, J.; Gustafsson, J. Å. *Endocrinology* **1997**, *138*, 863–870.
- Sato, M.; Grese, T. A.; Dodge, J. A.; Bryant, H. U.; Turner, C. H. *J. Med. Chem.* **1999**, *42*, 1–24.
- Nasr, A.; Breckwoldt, M. *Gynecol. Endocrinol.* **1998**, *12*, 43–59.
- Ulmsten, U. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 2–5.
- Cauley, J. A.; Cummings, S. R.; Black, D. M.; Mascioli, S. R.; Seeley, D. G. *Am. J. Obstet. Gynecol.* **1990**, *163*, 1438–1444.
- Blizzard, T. A.; Morgan, J. D., II; Mosley, R. T.; Birzin, E. T.; Frisch, K.; Rohrer, S. P.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2003**, 479–483.
- (a) Kuiper, G. C.; Carlsson, B.; Enmark, E.; Peltou-Huikko, M.; Nilsson, S.; Gustafsson, J. Å. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925–5930; (b) Mosselmann, S.; Polman, J.; Dijkema, R. *FEBS Lett.* **1996**, *392*, 49–53.
- Jordan, V. C. *J. Am. Chem. Soc.* **2003**, *125*, 1081.
- Jordan, V. C. *Br. J. Pharmacol.* **1993**, *3*, 495.
- Robinson, D. C.; Bloss, J. D.; Schiano, M. A. *Gynecol. Oncol.* **1995**, *59*, 186–190.
- Black, L. J.; Sato, M.; Rowley, E. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffman, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, J. D.; Bryant, H. U. *J. Clin. Invest.* **1994**, *93*, 63–69.
- Rosati, R. L.; Da Silva-Jardine, P.; Cameron, K. O.; Thompson, D. D.; Ke, H. Z.; Toler, S.; Brown, T. A.; Pan, L. C.; Ebbinghaus, C. F.; Reinhold, A. R.; Elliot, N. C.; Newhouse, B. N.; Tjoa, C. M.; Sweetnam, P. M.; Cole, M. J.; Arriola, M. W.; Gauthier, J. W.; Crawford, D. T.;

- Nickerson, D. F.; Pirie, C. M.; Qi, H.; Simmons, H. A.; Tkalcevic, G. T. *J. Med. Chem.* **1998**, *41*, 2928.
13. Nagarajan, K.; Talwalker, P. K.; Kulkarni, C. L.; Shah, R. K.; Shenoy, S. J.; Prabhu, S. S. *Indian J. Chem., Sect. B: Org. Chem. Including Med. Chem.* **1985**, *24B*(1), 83–97.
14. Renaud, J.; Bischoff, S. F.; Buhl, T.; Floersheim, P.; Fournier, B.; Halleux, C.; Kallen, J.; Keller, H.; Schlaeppli, J. M.; Stark, W. J. *Med. Chem.* **2003**, *46*, 2945.
15. Leake, R. E.; Habib, F. In *Steroid Hormone Receptors a Practical Approach*; Green, B., Leake, R. E., Eds.; IRL: Oxford, 1990; pp 67–92, Competition binding assay. The ability of various compounds to inhibit [³H]-estradiol binding was measured by a competition binding assay using dextran-coated charcoal. 293T cell extracts expressing either hER α or hER β were incubated in the presence of increasing concentrations of competitor and a fixed concentration of [³H]-estradiol (141 Ci/mmol, New England Nuclear, Boston, MA) in 50 mM TrisHCl pH 7.4, 1.5 mM EDTA, 50 mM NaCl, 10% glycerol, 5 mM DTT, 0.5 mg/mL β -lactoglobulin in a final volume of 0.2 mL. All competitors were dissolved in dimethylsulfoxide. The final concentration of receptor was 50 pM with 0.5 nM [³H]-estradiol. After 16 h at 4 °C, dextran-coated charcoal (20 μ L) was added. After 15 min at room temperature the charcoal was removed by centrifugation and the radioactive ligand present in the supernatant was measured by scintillation counting. All reagents were obtained from Sigma (St. Louis, MO).
16. Pike, A. C. W.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J. A.; Carlquist, M. *EMBO* **1999**, *18*, 4608–4618.
17. DNA Fluorometric Assay in 96-well Tissue Culture Plates Using Hoescht 33258 after Cell Lysis by Freezing in Distilled Water. Rago, R.; Mitchen, J.; Wilding, G. *Anal. Biochem.* **1990**, *191*, 31–34, Assay for estrogen receptor- α dependent proliferation of MCF7 cells. MCF7 human mammary epithelioid adenocarcinoma cells (American Type Culture Collection, Rockville, Maryland) were plated at 6000 cells/well in 96-well tissue culture plates in Iscove's Modified Dulbecco's Medium (Invitrogen, Carlsbad, California) supplemented with 5% fetal bovine serum and antibiotics. Following overnight attachment, cultures were incubated with two changes of medium plus 5% charcoal-dextran stripped fetal bovine serum ('stripped serum') over 48 h to deplete the cells of endogenous steroids. After stripping, cultures were incubated with fresh medium containing 5% stripped serum plus 0.1% dimethyl sulfoxide vehicle or test compounds in a final concentration of 0.1% dimethyl sulfoxide. Compounds were typically tested in log dilutions from 10⁻¹³ to 10⁻⁶ M in replicates of 8 wells. To assess proliferation-stimulating activity, medium and test compounds were replaced after 4 days and the DNA content of cultures was determined by Hoescht 33258 binding¹ after a total of 7 days exposure to test compounds. Alternatively, test compounds were added in the presence of 10⁻¹⁰ M 17- β estradiol to assess antagonism of estrogen-dependent proliferation. Estrogen antagonist activity was similarly determined by Hoescht dye binding after only 4 days of compound exposure.
18. Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. *Nature* **1997**, *389*, 753–758.