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Original article

Phenethyl pyridines with non-polar internal substitutents as selective ligands for estrogen receptor beta

Michael Waibel [a], Karen J. Kieser [a], Kathryn E. Carlson [a], Fabio Stossi [b], Benita S. Katzenellenbogen [b], John A. Katzenellenbogen [a,] *

[a] *Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, IL 61801, United States*
[b] *Department of Molecular and Integrative Physiology, University of Illinois, 600 South Matthews Avenue, Urbana, IL 61801, United States*

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ABSTRACT

To create estrogen receptor beta (ERβ)-selective ligands with improved biological characteristics, we have extended our investigations of structurally simple bibenzyl-core ligands by preparing a series of compounds in which one phenol is replaced by a 3-hydroxypyridine ring. These phenethyl pyridines were obtained by picoline anion alkylation, and compounds with different patterns of alkyl substitution on the central two carbon units were prepared. Binding affinities for ERα and ERβ were determined, and ligands with promising affinities and selectivities for ERβ were further tested for their gene transcriptional activity. Several compounds had high affinity selectivity and good potency selectivity in transcription assays. This study advances our understanding of compounds having ER-subtype selectivity and will help to direct efforts in developing novel ER ligands.

1. Introduction

Estrogens, which act through the estrogen receptors (ERs), regulate physiological processes not only in female reproductive tissues [1], but also in the skeletal [2], cardiovascular [3] and central nervous systems [4] of both males and females. While estrogen pharmaceuticals can provide health benefits in some tissues, they also promote cancer in other sites, such as the breast and the uterus [5,6]. It has proved possible, nevertheless, to create selective estrogen receptor modulators (SERMs), which are compounds that activate the ER selectively only in those tissues where estrogen action is desired, thus supporting estrogen health benefits, while minimizing the risk of cancer [7]. Tamoxifen [8] and raloxifene [9] are well known compounds that show such tissue-dependent estrogen agonist or antagonist effects, and they are currently being used in menopausal hormone replacement therapy to maintain bone mineral density [2] and for the prevention of breast cancer in women at high risk [10]. The tissue selectivity of these SERMs is thought to result from conformational characteristics of the ER-ligand complexes that exploit differences in their interactions with cell- and gene-specific coregulatory factors [7,11].

The ER system consists of two subtypes, ERα and ERβ, which have different patterns of tissue distribution and transcriptional regulation [12–14], and ERβ has proven to have antiproliferative effects in breast cancer cells when it is present together with ERα [15,16]. Although the amino acid sequence identity of ERα and ERβ ligand binding domains is only 58%, the residues lining the ligand binding pockets are almost identical. Of the ca. 25 residues lining the pocket, only two are different (Met421 and Leu384 in ERα are replaced by Ile373 and Met336 in ERβ); also, the internal volume of the ligand pocket in ERβ is about 100 Å^3 smaller than that of ERα [17]. This high sequence identity in the binding pockets of the two ERs creates a challenge in designing highly beta-selective ligands.

The phytoestrogen genistein **2** (Fig. 1) has a 20-fold binding selectivity relative to estradiol **1** [12], and we have described a number of ERβ-selective ligands [18–22], for example, the 70-fold selective diarylpropionitrile **3** (DPN) [22]. Others have reported ERβ-selective benzothiazoles [23], benzimidazoles [24] and

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Abbreviations: DPN, diarylpropionitrile; ER, estrogen receptor; LDA, lithium diisopropylamide; RBA, relative binding affinity; RTP, relative transcriptional potency; SERM, selective estrogen receptor modulator; TBAF, tetrabutylammonium fluoride; TBS, *t*-butyldimethylsilyl.

* Corresponding author. Tel.: +1 217 333 6310; fax: +1 217 333 7325.
E-mail address: jkatzene@uiuc.edu (J.A. Katzenellenbogen).

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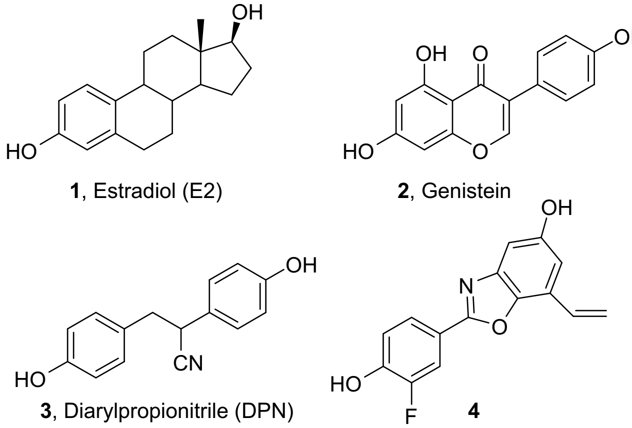


1, Estradiol (E2)

2, Genistein

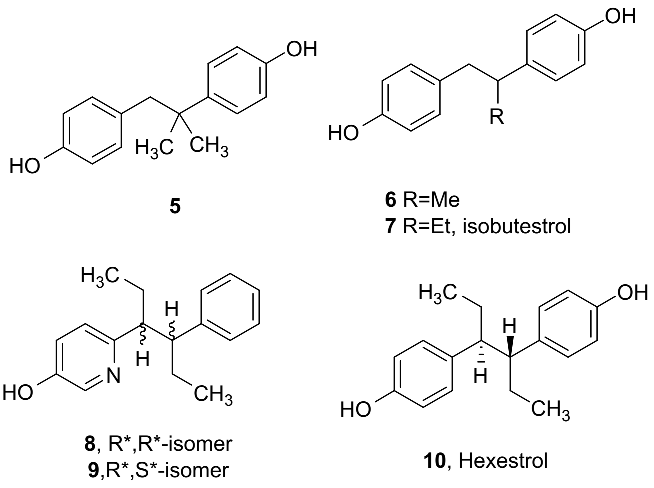
3, Diarylpropionitrile (DPN)

4

Fig. 1. Important ER ligands.

molecules having other types of heterocyclic cores [25–27], in particular, the benzoxazole **4**, with a 200-fold binding preference for ERβ [25].

We recently observed that bibenzyl-core compounds having small, non-polar internal substituents showed unexpectedly high selectivity for ERβ in terms of binding and transcriptional potency [28]. The most selective ligand of this series was the gem-dimethylated compound **5** (Fig. 2), but monoalkylated analogs that are homologs of isobutestrol **7** [20,29], such as ligand **6**, also showed good ERβ selectivity. This finding, that simple bisphenols with non-polar internal substituents were ERβ selective, was surprising, because most ERβ-selective non-steroidal ligands described previously have distinctly more polar interior substituents [20–27,30], as is illustrated by the ligands **2**–**4** in Fig. 1. We, therefore, thought that replacing one of the phenols of the bibenzyl core by a 3-hydroxypyridine ring, which would reintroduce an interior polar element into these compounds, might lead to a further increase in their ERβ selectivity. In addition, we recently reported related pyridine compounds **8** and **9**, analogs of hexestrol **10** [20], with both linker carbons bearing one ethyl chain. These compounds showed somewhat promising ERβ selectivity, but they had quite low affinity for both ERs, presumably because they were missing the second hydroxyl function, which is considered important for high affinity ER binding [31]. Therefore, we focused on making the pyridinol analogs having two hydroxyl groups.



5

6 R=Me
7 R=Et, isobutestrol

8, R*,R*-isomer
9,R*,S*-isomer

10, Hexestrol

Fig. 2. ER ligands having either a bibenzyl- or a phenethyl pyridyl core.

2. Chemistry

2.1. Synthesis of α-substituted phenethyl pyridines

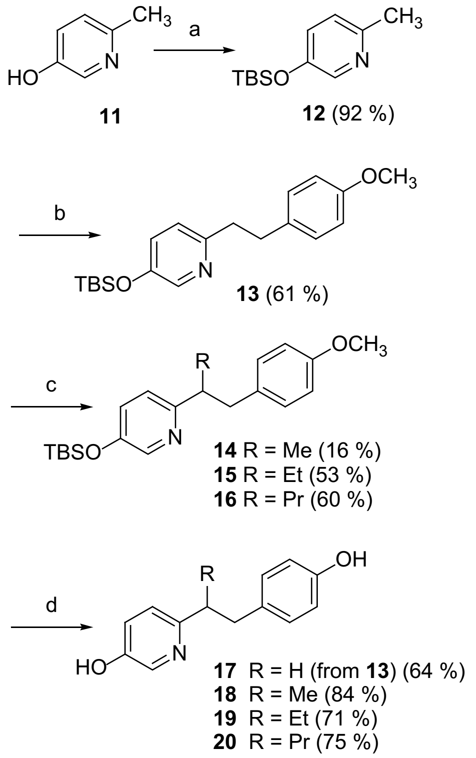
Scheme 1 shows the syntheses of phenethyl pyridines bearing different substituents on the linker carbon next to the pyridine ring. The reaction sequences started with the protection of 5-hydroxy-2-methyl pyridine **11**, as previously described [32]. The bulky *t*-butyldimethylsilyl (TBS)-group was chosen to prevent lithiation of the rather acidic C-6 position of the pyridine ring in the subsequent treatments with LDA. The methyl group of pyridine **12** was smoothly lithiated with LDA at low temperatures, and the anion was reacted with 4-methoxybenzyl chloride [20], yielding phenethyl pyridine **13**. Lithiation at the same position was repeated in the next step, followed by reaction with alkyl halides, producing compounds **14**–**16**. We attempted to improve the low yield of compound **14** by employing different alkylating agents: Use of methyl triflate gave only a very polar compound, possibly the N-methylated product; by contrast, use of methyl bromide or methyl iodide gave the desired compound in low yield, but a large amount of starting material could be recovered. Yields of **14** could be improved simply by repeating the reaction on recovered starting material.

Deprotection of compounds **13**–**16** was first attempted in two steps, by reacting them first with tetrabutylammonium fluoride (TBAF) to remove the TBS-group, followed by treatment with BBr_3 in CH_2Cl_2 to cleave the phenol ether. The mono deprotected compounds resulting from the first reaction, however, were highly polar and insoluble in CH_2Cl_2. They could be dissolved in DMSO or acetic acid, but no deprotection took place when they were refluxed with NaCN in DMSO for several days. On the other hand, refluxing with hydriodic acid in acetic [29] gave good yields of the target compounds **17**–**20** in short reaction times. In addition, we found that the TBS ether function of compounds **13**–**16** was also cleaved under these conditions. Thus, compounds **17**–**20** could be obtained from molecules **13**–**16** in one single step.

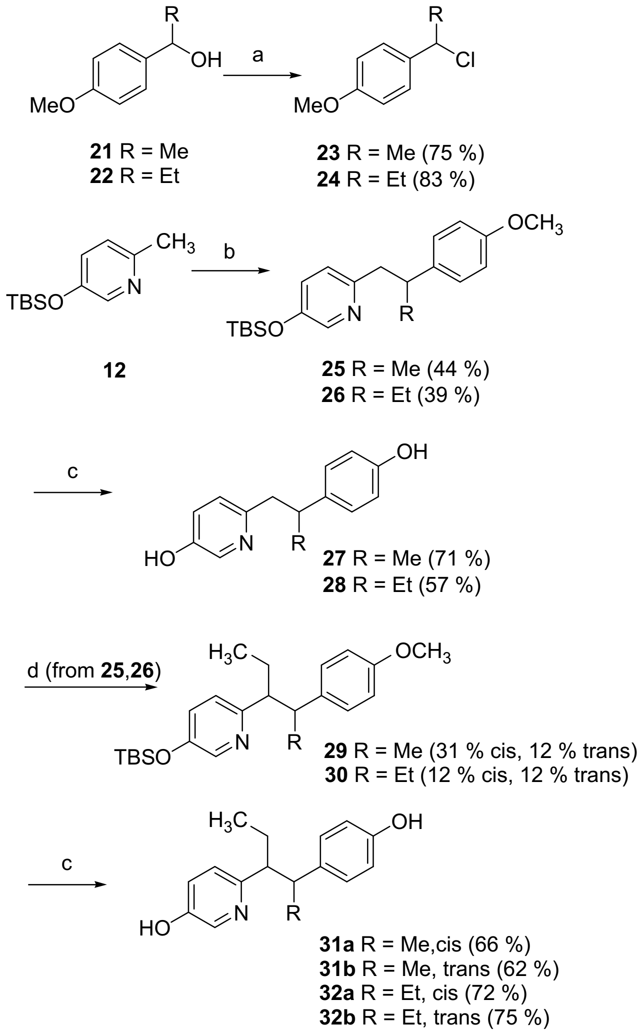
We also attempted to introduce two alkyl chains at the carbon next to the pyridine ring by alkylating twice. However, when compound **14** was treated with LDA, the deep purple color indicative of the lithiated pyridine, which was observed in all of the alkylations of compounds **12** and **13**, did not appear, and upon addition of the alkyl halide, no reaction took place. Employing the LIKOR and LIDAKOR superbases [33], which are reported to remove benzylic rather than aromatic protons, gave the same result. Our assumption that the pyridine methylene position in compound **14** is too sterically hindered to be deprotonated by these bases is supported by the fact that literature examples of such anion formations are only reported with less hindered systems [33–35]. Our attempts to use a stronger base, *n*-BuLi, gave a mixture of decomposition compounds, probably due to lithiation of the aromatic rings or addition to the pyridine ring.

2.2. Synthesis of β-substituted phenethyl pyridines

Scheme 2 illustrates the syntheses of phenethyl pyridines with either one alkyl chain at the benzylic position or one substituent on both linker carbons. In the first step, benzylic alcohols **21** and **22** were chlorinated using $SOCl_2$ to produce compounds **23** and **24** [36]. These reactions proceeded rapidly and in high yield at −78 °C to give the benzylic chlorides. They were then reacted with lithiated **12**, as described above in the preparation of **13**, to give phenethyl pyridines **25** and **26**. As before, deprotection using hydriodic acid gave the target compounds **27** and **28**. Further lithiation and ethylation of compounds **25** and **26** yielded the mixtures of diastereomers **29** and **30**. The target compounds **31a**, **31b**, **32a** and **32b**



Scheme 1. (a) Imidazole, TBS–Cl, DMF; (b) LDA, 4-(CH_3O)-$PhCH_2Cl$, THF; (c) LDA, RX (**14**: X = I; **15**, **16**: X = Br), THF; (d) HI, HOAc.



Scheme 2. (a) $SOCl_2$, CH_2Cl_2; (b) LDA, **23** (for **25**)/**24** (for **26**), THF; (c) HI, HOAc; (d) LDA, EtBr, THF.

could be obtained very efficiently, since it was possible to separate the diastereomers by chromatography after the final deprotection of **29** and **30**.

3. Pharmacology

3.1. Estrogen receptor binding assays

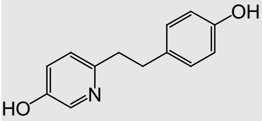
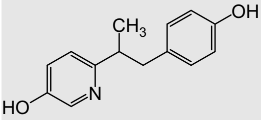
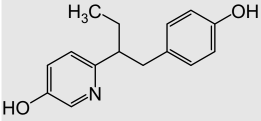
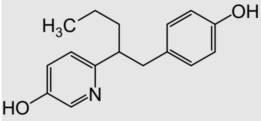
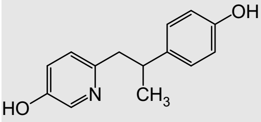
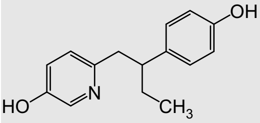
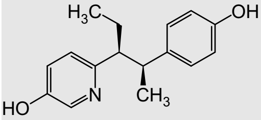
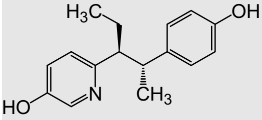
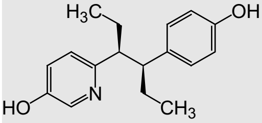
The phenethyl pyridines were tested in competitive radiometric binding assays, using purified full-length human ERα and ERβ, to determine their binding affinities for the two ERs [37,38]. The binding affinities are expressed relative to that of estradiol, which is set to 100%, to give relative binding affinity (RBA) values. Table 1 shows the RBA values of the synthesized pyridines and additionally the bibenzyls **6** and **7**, as well as hexestrol **10** and phenethyl pyridines **8** and **9**. *C* Log *P* values are also listed in Table 1.

The most ERβ-selective phenethyl pyridines were among the ones bearing one alkyl chain on the linker carbon next to the pyridine ring, namely compounds **18** (54-fold) and **19** (80-fold). Both **18** and **19** were more ERβ selective than their corresponding bibenzyl analogs, compound **6** (29-fold) and isobutestrol **7** (18-fold) [28]. This result confirmed our expectations and is consistent with the general finding that ERβ selectivity can be achieved with ligands, such as the phenethyl pyridines, that are more polar on the interior than the bibenzyls. However, although the phenethyl pyridines were more ERβ selective in their binding, their overall affinity was almost 100-fold less than that of the corresponding bibenzyls.

The general trend in this first compound series is that both affinity and selectivity for ERβ increase with the chain length of the substituents, reaching a maximum at two carbon atoms (**17** vs. **18** vs. **19**); further extension of the chain lowered both affinity and selectivity (**20**). Interestingly, the trend shown by **17**–**19** differs from the more general finding that increased substitution results in higher affinities but lower selectivity, a behavior illustrated by isobutestrol **7** compared to hexestrol **10** [20], as well as by their stilbene analogs [28]. Placing the alkyl substituent on the benzylic carbon also resulted in compounds having quite good ERβ selectivities, but these were lower than those of the first compound series (**27** vs. **18**, **28** vs. **19**). Curiously, at the benzylic position, methyl substitution gave better affinity and selectivity than ethyl substitution (**18**, **19** vs. **27**, **28**).

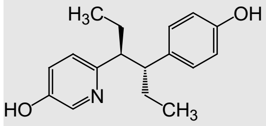
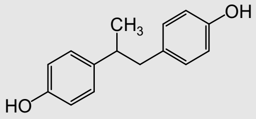
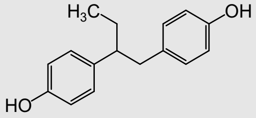
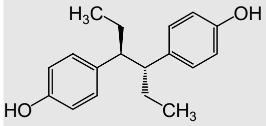
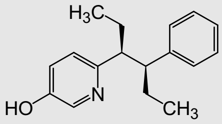
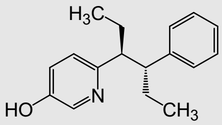
The fact that compound **19** showed such good selectivity, together with the observed trend that higher substitution resulted in both increased affinity and selectivity, led us to examine whether the affinity and selectivity of **19** might be improved by introducing a second alkyl chain, on the benzylic position. The anti (R^*,S^*) isomers of both doubly substituted compounds (**31b**, **32b**) showed markedly increased affinity as desired, but selectivity was reduced in comparison to their monosubstituted analogs (**31b** vs. **19**, **27**; **32b** vs. **19**, **28**). On the other hand, the *syn* (R^*,R^*) isomers (**31a**, **32a**) had much lower affinity than the corresponding anti-isomers (**31a** vs. **31b**, **32a** vs. **32b**). This trend is also known for the *meso* (R^*,S^*) and dl (R^*,R^*) diastereomers of hexestrol [29], as well as for the monohydroxy reference compounds **8** and **9**, studied earlier [20].

Table 1
ERα and ERβ relative binding affinity (RBA), relative transcriptional potency (RTP), transcriptional activity (EC_{50} values), and affinity and potency selectivities for phenethyl pyridines and related ligands.

Ligand	Ligand binding				Transcription potency					
	RBA[a] (%)		β/α affinity ratio[b]	C Log P[c]	RTP[d] (%)		β/α potency ratio[b]	EC_{50}[e] (nM)		β/α potency ratio[b]
	ERα	ERβ			ERα	ERβ		ERα	ERβ	
 **17**	<0.004	0.025 ± 0.006	6	2.71	ND[f]	ND	ND	NT[f]	NT	NT
 **18**	<0.005	0.27 ± 0.07	54	3.11	0.03	0.83	28	330	60	5.5
 **19**	0.009 ± 0.003	0.72 ± 0.05	80	3.64	0.15	2.94	20	65	17	3.8
 **20**	0.021	0.101 ± 0.03	5	4.17	ND	ND	ND	NT	NT	NT
 **27**	<0.006	0.17 ± 0.02	28	3.11	0.023	0.29	13	440	170	2.6
 **28**	0.018 ± 0.001	0.15 ± 0.02	8	3.64	ND	ND	ND	NT	NT	NT
 **31a**	0.016 ± 0.004	0.119 ± 0.002	7	4.04	ND	ND	ND	NT	NT	NT
 **31b**	0.7 ± 0.2	5.3 ± 1.3	7	4.04	91	45	0.5	0.11	1.1	0.1
 **32a**	0.035 ± 0.006	0.67 ± 0.14	19	4.57	0.63	1.9	3.0	16	26	0.6

(continued on next page)

Table 1 (*continued*)

Ligand	Ligand binding				Transcription potency					
	RBA[a] (%)		β/α affinity ratio[b]	C Log P[c]	RTP[d] (%)		β/α potency ratio[b]	EC_{50}[e] (nM)		β/α potency ratio[b]
	ERα	ERβ			ERα	ERβ		ERα	ERβ	
 **32b**	3.3 ± 0.7	7.5 ± 1.6	2	4.57	91	82	0.9	0.11	0.61	0.2
 **6**	0.71 ± 0.08	20.5 ± 5.0	29	3.65	1.5	42	27	6.5	1.2	5
 Isobutestrol, **7**	2.2 ± 0.4	39.5 ± 8.4	18	4.18	ND	ND	ND	NT	NT	NT
 Hexestrol, **10**	277 ± 57	697 ± 194	2.5	5.11	ND	ND	ND	NT	NT	NT
 **8**[g]	0.010	0.023	2	5.23	ND	ND	ND	NT	NT	NT
 **9**[g]	0.081	0.684	8	5.23	ND	ND	ND	NT	NT	NT

[a] Relative binding affinity (RBA) values are determined by competitive radiometric binding assays and are expressed as $EC_{50}^{[\text{estradiol}]}/EC_{50}^{[\text{compound}]} \times 100$ (RBA, estradiol = 100). In these assays, the K_d for estradiol is 0.2 nM for ERα and 0.5 nM for ERβ. For details, see Experimental section.

[b] For each set of affinity or potency values, the β/α ratio is calculated such that the ratio is >1 for compounds having higher affinity or potency on ERβ than on ERα.

[c] *C* Log *P* values are calculated within ChemDraw Ultra 7.0.3.

[d] Relative transcriptional potency (RTP) values are expressed as $EC_{50}^{(\text{estradiol})}/EC_{50}^{(\text{ligand})} \times 100$ (RTP, estradiol = 100). In these assays, the EC_{50} for estradiol is 0.1 nM for ERα and 0.5 nM for ERβ.

[e] Transcriptional activity was measured using a cotransfection assay in human endometrial cancer (HEC-1) cells (see Experimental section and Fig. 3). Transcriptional potency = EC_{50}.

[f] ND = not determined; NT = not tested.

[g] Data are from De Angelis et al. [20].

The lower binding affinity of the monohydroxy phenethyl pyridines **8** and **9** (prepared previously [20]) compared to the corresponding dihydroxy analogs **32a** and **32b** is notable. It also suggests that these two ligand sets might be binding to ER in opposite directions: The pyridinol ring of **8** and **9** most probably mimics the A-ring of estradiol, and binding is rather poor because the more polar ring has a less favorable interaction with Phe356 which is near the ligand A-ring in ERβ; with the isomers **32**, on the other hand, a phenol could be acting as an A-ring mimic, with consequently higher affinity due to a more favorable interaction of this less polar ring with Phe356 in ERβ. Interestingly, with most non-steroidal estrogens, a second hydroxyl group seems important for high affinity binding [31].

Also of note is the modest affinity of all pyridine derivatives compared to the binding of their bibenzyl analogs. Because the pyridine derivatives are more polar than the bibenzyls (compare estimated *C* Log *P* values, Table 1: **18**, **27** vs. **6**; **19**, **28** vs. **7**; **32a**, **32b** vs. **10**), it seems reasonable that the pyridines would experience a greater desolvation energy penalty upon moving from water to the ligand binding pocket of the ERs, thus disfavoring the binding process energetically. This desolvation energy penalty experienced by polar ER ligands is evident in other ligand series: The moderately polar alkyl-triaryl-pyrazoles bind much better to ERα than their isostructural but more polar alkyl-triaryl-imidazoles [39], but less well than their isostructural but less polar furans [40]. Similarly, the pyridine derivatives of deoxyhexestrol bind better than their more

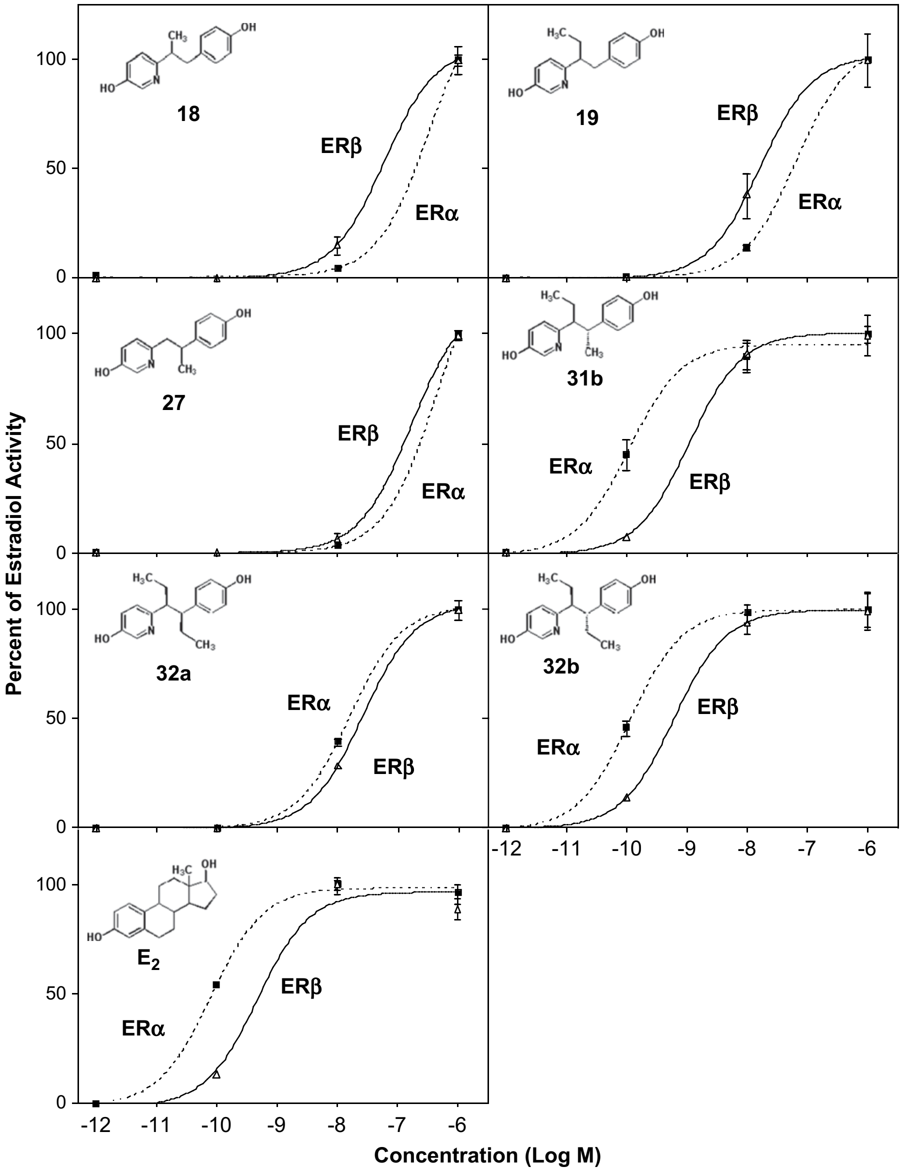


Fig. 3. Transcriptional efficacy of ligands **18**, **19**, **27**, **31b**, **32a** and **32b** on ERα and ERβ at 0.1, 10, and 1000 nM, with estradiol (E_2) for comparison (lowest left panel). The diagrams include as well the values corresponding to a ligand concentration of 0 M (V = negative control). Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα and ERβ and the estrogen-responsive gene 2ERE-pS2-Luc, and were incubated with the indicated concentrations of ligand for 24 h. Luciferase activity and β-galactosidase activity were assayed as described [41]. Estradiol activity at 10 nM is set at 100%.

polar pyrimidine analogs, consistent with the higher *C* Log *P* values of the former system [20].

While it is notable that both anti-isomers, **31b** and **32b**, are less polar (based on their chromatographic behavior) than the corresponding *syn*-isomers, **31a** and **32a**, which might account for their higher affinity, the markedly higher ER binding affinity of anti-bibenzyl diols is thought to arise from their preference to adopt a biaryl anti conformation, preferred by the ER ligand binding pocket, rather than a *syn* conformation [29].

3.2. *Activity on gene transcription*

The transcriptional activities of six selected compounds (**18**, **19**, **27**, **31b**, **32a**, **32b**) that showed good ERβ binding selectivity were tested for their agonistic character as regulators of gene transcription, together with estradiol. They were assayed in human endometrial cancer (HEC-1) cells, transfected with expression plasmids for ERα or ERβ and an estrogen-responsive reporter gene [41]. The dose–response curves for these compounds are given in Fig. 3, and the EC_{50} values are listed in Table 1. This table also includes, for comparison, the EC_{50} values for other related compounds determined in a recent study [28].

To facilitate comparisons of the ER subtype *transcriptional potencies* of our compounds with their ER subtype *binding affinities*, the EC_{50} values from the transcription assays were converted to relative transcriptional potency (RTP) values (See Table 1, footnote d). The RTP values provide a measure of transcriptional potency relative to that of estradiol and thus can be better compared to their binding affinities, which are also measured relative to estradiol by the competitive radiometric binding assays. Estradiol has a 2.5-fold preference in favor of ERα in terms of binding (K_d [ERα] = 0.2 nM vs. [ERβ] = 0.5 nM) and a 5-fold preference in terms of transcriptional potency (EC_{50} [ERα] = 0.1 nM vs. [ERβ] = 0.5 nM).

As shown in Fig. 3 and listed in Table 1, the three compounds having the highest ERβ relative binding selectivity (**18**, **19**, and **27**) also showed good ERβ selectivity in terms of transcriptional potency. In fact, the β/α ratio of their RBA values and their RTP values is quite comparable. The other three compounds (**31b**, **32a**, and **32b**), which have generally lower ERβ relative binding selectivity, are also less ERβ selective in terms of their RTP values. Compound **31b**, in fact, has an ERα-potency preference that is greater than that of estradiol; **32b** is similar to that of estradiol, but **32a** is more ERβ selective than estradiol. Irrespective of their ER-subtype selectivities, the higher absolute affinities of the anti disubstituted compounds (**31b** and **32b**) compared to the others (**18**, **19**, **27**, **31a**, and **32a**) are reflected in their overall much higher transcriptional potencies. Compounds **31b** and **32b**, in particular, were found to be extremely potent, thus being the most potent ligands of the whole series, although they are not at all ERβ selective.

4. Conclusion

This study extends our investigation of simple, non-steroidal ERβ-selective bibenzyl diol ligands having non-polar internal substituents to their corresponding phenethyl pyridinols. The nitrogen-for-carbon substitution in one ring, which transforms a phenol into a pyridinol and makes the interior of the ligand more polar, gives ligands that conform more closely to the “narrow but somewhat polar interior” that characterizes the pharmacophore of most ERβ-selective ligands reported to date. Some of the monosubstituted phenethyl pyridinols did exhibit high ERβ-selective relative binding affinity and transcriptional potency that in some cases was greater than that of the corresponding bibenzyl diols, but their overall binding affinities were much lower. This reduction in affinity probably comes from the greater desolvation penalty experienced by the more polar pyridine compounds, which tracks with their lower lipophilic character as measured by their lower *C* Log *P* values. In reporter gene transcription assays, the ERβ-selective transcriptional potencies of the phenethyl pyridinols mirrored quite closely their ERβ-selective binding affinities. The vicinally disubstituted phenethyl pyridinols, like the disubstituted bibenzyl diols, showed enhanced ER binding affinity and transcriptional potencies, but reduced ERβ selectivities.

Unlike other ERβ-selective ligands thus far described, polar elements in the interior region of these structurally simple bibenzyl diol and phenethyl pyridinol ligands are not required for their ER-subtype selectivity; in fact, while introducing the polar nitrogen into a benzene to make a pyridine maintained ERβ selectivity, it did not improve it, and it uniformly reduced binding affinity. Thus, polar interior elements are not a prerequisite for non-steroidal ERβ-selective ligands. Our findings provide further understanding of the design elements that should be considered in the continuing efforts to create ERβ-selective ligands with improved biological characteristics.

5. Experimental section

5.1. Materials and methods

Reagents and solvents were purchased from Aldrich and Fisher Scientific. THF and dichloromethane were dried using a solvent-dispensing system (SDS) (neutral alumina columns) built by J.C. Meyer on the basis of a design developed by Pangborn et al. [42]. Glassware was oven- or flame-dried, assembled while hot, and cooled under nitrogen atmosphere. All reactions were performed in anhydrous solvents and under nitrogen atmosphere, unless stated otherwise. Reaction progress was monitored by thin-layer chromatography (TLC) using 0.25 mm Merck silica gel 60 glass plates containing F_{254} UV-Indicator. The plates were visualized by either UV light (254 nm), or dipping in a solution of potassium permanganate followed by heating. Column chromatography was performed using Woelm 32–63 μm silica gel packing. ^{1}H and ^{13}C NMR were recorded on Varian UNITY 400 or 500 MHz spectrometers. Chemical shifts are reported in ppm and referenced from solvent references. NMR coupling constants are reported in hertz. Mass spectra were obtained on a Micromass 70-VSE spectrometer (EI, 70 eV; CI, methane). Melting point (mp) determinations were carried out on Thomas Hoover Unimelt capillary apparatus and are uncorrected. All target compounds shown in Scheme 1 could be recrystallized, while all compounds in Scheme 2 could not. They were obtained as oils that spontaneously foamed upon drying to form amorphous solids, which might explain their less distinct melting points. Elemental analyses were performed by the Microanalysis Service Laboratory of the University of Illinois. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

5.2. Chemical synthesis

5.2.1. 5-(tert-Butyl-dimethyl-silanyloxy)-2-[2-(4-methoxy-phenyl)-ethyl]-pyridine (13)

Lithium diisopropylamide (LDA) (10.5 mL of a 2 M solution in heptane/THF/ethylbenzene) was added to 3.5 mL THF at −78 °C, and then compound **12** (4.00 g, 17.94 mmol, dissolved in 3.5 mL THF) was added dropwise over a time period of 15 min. After 45 min, 1-chloromethyl-4-methoxy-benzene (2.91 mL, 21.5 mmol, dissolved in 3.5 mL THF) was added in the same manner as **12** and the reaction was stirred for 1 h. The mixture was allowed to go to rt during a 1.5 h time period and stirred for 1 h at rt. The reaction was quenched by adding NH_4Cl (sat. aqueous solution) and extracted three times with EtOAc. The organic extracts were dried over Na_2SO_4 and the solvent was removed under vacuum. After purification by flash chromatography (10% EtOAc/hexanes), **13** was obtained as a colorless oil (3.73 g, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 2.8, 1H), 7.08 (d, *J* = 8.7, 2H), 7.03 (dd, *J* = 8.4, 2.8, 1H), 6.92 (d, *J* = 8.4, 1H), 6.80 (d, *J* = 8.7, 2H), 3.78 (s, 3H), 3.01–2.92 (m, 4H), 0.98 (s, 9H), 0.20 (s, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 157.77, 153.91, 150.21, 141.55, 133.78, 129.38, 127.28, 123.17, 113.68, 55.19, 39.55, 35.29, 25.58, 18.17, −4.51; MS (EI) *m*/*z* 343 (M^+, 23).

5.2.2. General alkylation procedure

LDA was added to THF at −78 °C, and then the starting material **13** (1 eq, dissolved in THF) was added dropwise over a time period of 15 min. After 45 min, the alkyl halide (dissolved in THF) was added, and after further 45 min pure alkyl halide was added, both times the addition was done over a 15 min time period. The mixture was allowed to go to rt during a 1.5 h time period and stirred for 45 min at rt. The reaction was quenched by adding NH_4Cl (sat. aqueous solution) and extracted three times with EtOAc. The organic extracts were dried over Na_2SO_4 and the solvent was removed under vacuum, and the crude product was purified by flash chromatography.

5.2.3. 5-(tert-Butyl-dimethyl-silanyloxy)-2-[2-(4-methoxy-phenyl)-1-methyl-ethyl]-pyridine (14)

According to the general alkylation procedure, lithiation of **13** was performed by adding **13** (890 mg, 2.6 mmol, in 0.5 mL THF) to a solution of LDA (1.5 mL of a 2 M solution in heptane/THF/ethylbenzene) in 1 mL THF. Adding methyl iodide (1st addition: 487 μL, 7.8 mmol, in 0.5 mL THF, 2nd addition: 487 μL pure) to a stirred solution of lithiated **13** gave **14** as a colorless oil (149 mg, 16%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, *J* = 2.9, 1H), 7.00 (dd, *J* = 8.4, 2.9, 1H), 6.95 (d, *J* = 8.7, 2H), 6.86 (d, *J* = 8.4, 1H), 6.74 (d, *J* = 8.7, 2H), 3.75 (s, 3H), 3.09–3.03 (m, 1H), 2.99–2.95 (m, 1H), 2.77–2.73 (m, 1H), 1.25 (d, *J* = 6.9, 3H), 0.98 (s, 9H), 0.20 (s, 6H); ^{13}C NMR (500 MHz, $CDCl_3$)

δ 158.09, 157.68, 150.16, 141.33, 132.81, 130.01, 127.21, 122.16, 113.42, 55.12, 43.05, 42.68, 25.58, 19.93, 18.17, −4.50; MS (CI) *m*/*z* 358 ($M^{+}+1$, 72).

5.2.4. 5-(*tert*-Butyl-dimethyl-silanyloxy)-2-[1-(4-methoxy-benzyl)-propyl]-pyridine (*15*)

According to the general alkylation procedure, lithiation of 890 mg of **13** was performed as described in the case of compound **14**. Adding ethyl bromide (1st addition: 233 μL, 3.1 mmol, in 0.5 mL THF, 2nd addition: 233 μL pure) to a stirred solution of lithiated **13** gave **15** as a colorless oil (507 mg, 53%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (d, $J=2.8$, 1H), 6.97 (dd, $J=8.5$, 2.8, 1H), 6.90 (d, $J=8.8$, 2H), 6.77 (d, $J=8.5$, 1H), 6.71 (d, $J=8.8$, 2H), 3.74 (s, 3H), 2.95–2.76 (m, 3H), 1.78–1.66 (m, 2H), 0.98 (s, 9H), 0.76 (t, $J=7.4$, 3H), 0.20 (s, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 157.57, 156.64, 150.10, 141.53, 132.92, 129.96, 126.91, 123.52, 113.35, 55.09, 50.86, 41.17, 27.51, 25.58, 18.18, 12.06, −4.50; MS (CI) *m*/*z* 372 ($M^{+}+1$, 100).

5.2.5. 5-(*tert*-Butyl-dimethyl-silanyloxy)-2-[1-(4-methoxy-benzyl)-butyl]-pyridine (*16*)

According to the general alkylation procedure, lithiation of 890 mg of **13** was performed as described in the case of compound **14**. Adding propyl bromide (1st addition: 283 μL, 3.1 mmol, in 0.5 mL THF, 2nd addition: 283 μL pure) to a stirred solution of lithiated **13** gave **16** as a colorless oil (605 mg, 60%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, $J=2.8$, 1H), 6.96 (dd, $J=8.4$, 2.8, 1H), 6.88 (d, $J=8.8$, 2H), 6.76 (d, $J=8.4$, 1H), 6.70 (d, $J=8.8$, 2H), 3.74 (s, 3H), 2.93–2.81 (m, 3H), 1.77–1.69 (m, 1H), 1.65–1.58 (m, 1H), 1.19–1.10 (m, 2H), 0.98 (s, 9H), 0.83 (t, $J=7.3$, 3H), 0.20 (s, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 157.57, 156.89, 150.08, 141.48, 132.91, 129.95, 126.95, 123.42, 113.34, 55.10, 48.90, 41.45, 36.89, 25.58, 20.66, 18.18, 14.08, −4.50; MS (CI) *m*/*z* 386 ($M^{+}+1$, 100).

5.2.6. General deprotection procedure

A solution of starting material in acetic acid was added dropwise to a solution of hydriodic acid (57 wt.% in water) in acetic acid. The mixture was refluxed, cooled to rt as soon as deprotection was complete and diluted with water. By adding NaOH (10% aqueous solution) and $NaHCO_3$ (sat. aqueous solution), a slightly basic pH value was adjusted (buffer area of $NaHCO_3$). The mixture was extracted three times with EtOAc and the combined organic extracts were washed with $NaHSO_3$ (10% aqueous solution). The organic phase was dried over Na_2SO_4, the solvent was removed under vacuum, and purification was performed by flash chromatography.

5.2.7. 6-[2-(4-Hydroxy-phenyl)-ethyl]-pyridin-3-ol (*17*)

According to the general deprotection procedure, compound **13** (765 mg, 2.23 mmol) in 12 mL acetic acid was added to 3 mL hydriodic acid (57 wt.% in water) in 12 mL acetic acid and refluxed for 4 h. After reaction work up, purification by flash chromatography (pure EtOAc) and re-crystallization from EtOAc/hexane, **17** was obtained as a white solid (308 mg, 64%, mp 136 °C). ^{1}H NMR (500 MHz, acetone-d_6) δ 8.14 (d, $J=2.9$, 1H), 7.12 (dd, $J=8.4$, 2.9, 1H), 7.02–6.99 (m, 3H) 6.71 (d, $J=8.6$, 2H), 2.94–2.84 (m, 4H); ^{13}C NMR (500 MHz, acetone-d_6) δ 156.34, 153.11, 152.61, 137.90, 133.52, 130.14, 123.87, 123.28, 115.85, 40.09, 35.97; MS (EI) *m*/*z* 215 (M^{+}, 72). HRMS (EI) calcd for $C_{13}H_{13}NO_2$: 215.0946, found 215.0943. Anal. $C_{13}H_{13}NO_2\cdot 0.2H_2O$: Calcd. C, 71.34; H, 6.17; N, 6.30. Found C, 71.11; H, 6.09; N, 6.38.

5.2.8. 6-[2-(4-Hydroxy-phenyl)-1-methyl-ethyl]-pyridin-3-ol (*18*)

According to the general deprotection procedure, compound **14** (253 mg, 0.71 mmol) in 3.8 mL acetic acid was added to 955 μL hydriodic acid (57 wt.% in water) in 3.8 mL acetic acid and refluxed for 2.5 h. After reaction work up, purification by flash chromatography (pure EtOAc) and re-crystallization from EtOAc/acetone, **18** was obtained as a white solid (137 mg, 84%, mp 219 °C). ^{1}H NMR (500 MHz, acetone-d_6) 8.14 (d, $J=2.9$, 1H), 7.05 (dd, $J=8.4$, 2.9, 1H), 6.93 (d, $J=8.4$, 1H), 6.89 (d, $J=8.6$, 2H), 6.66 (d, $J=8.6$, 2H), 3.06–2.99 (m, 1H), 2.94–2.90 (m, 1H), 2.69–2.65 (m, 1H), 1.17 (d, $J=6.9$, 3H); ^{13}C NMR (500 MHz, acetone-d_6) δ 157.33, 156.27, 152.55, 137.86, 132.63, 130.77, 123.14, 122.95, 115.63, 43.76, 43.25, 20.65; MS (CI) *m*/*z* 230 ($M^{+}+1$, 100). HRMS (CI) calcd for $C_{14}H_{16}NO_2$: 230.1181, found 230.1181. Anal. $C_{14}H_{15}NO_2\cdot 0.1H_2O$: Calcd. C, 72.77; H, 6.63; N, 6.06. Found C, 72.73; H, 6.50; N, 6.12.

5.2.9. 6-[1-(4-Hydroxy-benzyl)-propyl]-pyridin-3-ol (*19*)

According to the general deprotection procedure, compound **15** (360 mg, 0.97 mmol) in 5.5 mL acetic acid was added to 1.4 mL hydriodic acid (57 wt.% in water) in 5.5 mL acetic acid and refluxed for 1.5 h. After reaction work up, purification by flash chromatography (75% EtOAc/hexanes) and re-crystallization from EtOAc/hexanes, **19** was obtained as a white solid (167 mg, 71%, mp 196 °C). ^{1}H NMR (500 MHz, acetone-d_6) 8.16 (d, $J=2.9$, 1H), 7.03 (dd, $J=8.4$, 2.9, 1H), 6.86–6.82 (m, 3H), 6.63 (d, $J=8.6$, 2H), 2.90–2.84 (m, 1H), 2.81–2.75 (m, 2H), 1.75–1.60 (m, 2H), 0.70 (t, $J=7.5$, 3H); ^{13}C NMR (500 MHz, acetone-d_6) δ 156.17, 155.74, 152.52, 138.07, 132.68, 130.73, 124.38, 122.82, 115.58, 51.50, 41.86, 28.42, 12.32; MS (EI) *m*/*z* 243 (M^{+}, 43). HRMS (EI) calcd for $C_{15}H_{17}NO_2$: 243.1259, found 243.1260. Anal. $(C_{15}H_{17}NO_2)\cdot 0.2H_2O$: Calcd. C, 73.08; H, 8.20; N, 5.33. Found C, 73.10; H, 7.46; N, 5.73.

5.2.10. 6-[1-(4-Hydroxy-benzyl)-butyl]-pyridin-3-ol (*20*)

According to the general deprotection procedure, compound **16** (216 mg, 0.56 mmol) in 5.7 mL acetic acid was added to 1.4 mL hydriodic acid (57 wt.% in water) in 5.7 mL acetic acid and refluxed for 2 h. After reaction work up, purification by flash chromatography (80% EtOAc/hexanes) and re-crystallization from EtOAc/hexanes, **20** was obtained as a white solid (108 mg, 75%, mp 157 °C). ^{1}H NMR (500 MHz, acetone-d_6) 8.15 (d, $J=2.9$, 1H), 7.01 (dd, $J=8.4$, 2.9, 1H), 6.85–6.82 (m, 3H), 6.62 (d, $J=8.6$, 2H), 2.92–2.84 (m, 2H), 2.79–2.73 (m, 1H), 1.75–1.68 (m, 1H), 1.59–1.52 (m, 1H), 1.16–1.03 (m, 2H), 0.79 (t, $J=7.4$, 3H); ^{13}C NMR (500 MHz, acetone-d_6) δ 156.17, 156.00, 152.47, 138.09, 132.69, 130.73, 124.23, 122.76, 115.57, 49.49, 42.18, 37.96, 21.25, 14.36; MS (EI) *m*/*z* 257 (M^{+}, 32). HRMS (EI) calcd for $C_{16}H_{19}NO_2$: 257.1416, found 257.1421.

5.2.11. 1-(1-Chloro-ethyl)-4-methoxy-benzene (*23*)

$SOCl_2$ (2.47 mL, 34.0 mmol) in 6 mL CH_2Cl_2 was added to a stirred solution of alcohol **21** (4.32 g, 28.4 mmol) in 20 mL CH_2Cl_2 at −78 °C over a time period of 15 min. The reaction was quenched with water at low temperature and extracted three times with EtOAc. The organic extracts were dried over Na_2SO_4 and the solvent was removed under vacuum. After distillation using a "Kugelrohr", **23** was obtained as a colorless oil (3.62 g, 75%, boiling at 90 °C/2.5 Torr). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, $J=8.8$, 2H), 6.88 (d, $J=8.8$, 2H), 5.10 (quart, $J=6.8$, 1H), 3.81 (s, 3H), 1.85 (d, $J=6.8$, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 159.34, 134.94, 127.74, 113.86, 58.76, 55.24, 26.37; MS (CI) *m*/*z* 135 ($M^{+}-35$, 100).

5.2.12. 1-(1-Chloro-propyl)-4-methoxy-benzene (*24*)

The reaction was performed as described above for **23**. Usage of $SOCl_2$ (3.94 mL, 54.24 mmol) in 10 mL CH_2Cl_2, and alcohol **22** (7.50 g, 45.2 mmol) in 30 mL CH_2Cl_2 gave **24** as a colorless oil (6.91 g, 83%, boiling at 95 °C/1.5 Torr) after purification by distillation. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, $J=8.8$, 2H), 6.89 (d, $J=8.8$, 2H), 4.79 (t, $J=7.3$, 1H), 3.81 (s, 3H), 2.21–2.03 (m, 2H), 1.00 (d, $J=7.3$, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 159.38, 133.92, 128.19, 113.86, 65.44, 55.22, 33.10, 11.75; MS (CI) *m*/*z* 149 (M$^+$ − 35, 100).

5.2.13. *5-(*tert*-Butyl-dimethyl-silanyloxy)-2-[2-(4-methoxy-phenyl)-propyl]-pyridine (**25**)*

The reaction was carried out as described above for the synthesis of compound **13**. Lithiation of **12** was performed by adding **12** (5.06 g, 22.70 mmol, in 4.2 mL THF) to a solution of LDA (13.62 mL of a 2 M solution in heptane/THF/ethylbenzene) in 4.2 mL THF. Adding **23** (3.86 g, 22.70 mmol, in 4.2 mL THF) to a stirred solution of lithiated **12** gave **25** as a yellow oil (3.57 g, 44%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.14 (d, $J = 2.8$, 1H), 7.08 (d, $J = 8.8$, 2H), 6.96 (dd, $J = 2.8$, 8.4, 1H), 6.79 (d, $J = 8.8$, 2H), 6.77 (d, $J = 8.4$, 1H), 3.77 (s, 3H), 3.21–3.14 (m, 1H), 2.96–2.86 (m, 2H), 1.23 (t, $J = 7.1$, 3H), 0.97 (s, 9H), 0.19 (s, 6H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 157.75, 153.35, 150.10, 141.47, 138.86, 127.87, 127.00, 123.80, 113.59, 55.15, 46.49, 39.69, 25.57, 21.40, 18.16, −4.53; MS (CI) *m*/*z* 358 (M$^+$ + 1, 100).

5.2.14. *5-(*tert*-Butyl-dimethyl-silanyloxy)-2-[2-(4-methoxy-phenyl)-butyl]-pyridine (**26**)*

The reaction was carried out as described above for the synthesis of compound **13**. Lithiation of **12** was performed by adding **12** (5.22 g, 23.41 mmol, in 4.3 mL THF) to a solution of LDA (14.00 mL of a 2 M solution in heptane/THF/ethylbenzene) in 4.3 mL THF. Adding **24** (4.30 g, 23.37 mmol, in 4.3 mL THF) to a stirred solution of lithiated **12** gave **26** as a yellow oil (3.38 g, 39%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.11 (d, $J = 2.8$, 1H), 6.99 (d, $J = 8.7$, 2H), 6.90 (dd, $J = 8.4$, 2.8, 1H), 6.76 (d, $J = 8.7$, 2H), 6.68 (d, $J = 8.4$, 1H), 3.76 (s, 3H), 3.04–2.98 (m, 1H), 2.91–2.84 (m, 2H), 1.74–1.66 (m, 1H), 1.64–1.55 (m, 1H), 0.96 (s, 9H), 0.77 (t, $J = 7.4$, 3H), 0.17 (s, 6H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 157.71, 153.43, 149.98, 141.39, 136.80, 128.60, 126.90, 123.82, 113.47, 55.09, 47.52, 45.00, 28.69, 25.57, 18.14, 12.05, −4.53; MS (CI) *m*/*z* 372 (M$^+$ + 1, 100).

5.2.15. *6-[2-(4-Hydroxy-phenyl)-propyl]-pyridin-3-ol (**27**)*

According to the general deprotection procedure, compound **25** (300 mg, 0.84 mmol) in 4.3 mL acetic acid was added to 1.14 mL hydriodic acid (57 wt.% in water) in 4.3 mL acetic acid and refluxed for 2 h. After reaction work up, purification by flash chromatography (80% ether/CH$_2$Cl$_2$) and additional purification by preparative TLC (80% ether/CH$_2$Cl$_2$), **27** was obtained as a white solid (136 mg, 71%, mp 152 °C). ^{1}H NMR (500 MHz, acetone-d_6) 8.10 (d, $J = 2.9$, 1H), 7.09 (dd, $J = 2.9$, 8.4, 1H), 7.03 (d, $J = 8.5$, 2H), 6.88 (d, $J = 8.4$, 1H), 6.71 (d, $J = 8.5$, 2H), 3.18–3.10 (m, 1H), 2.91–2.70 (m, 2H), 1.15 (d, $J = 6.9$, 3H); ^{13}C NMR (500 MHz, acetone-d_6) δ 156.36, 152.61, 152.48, 138.66, 137.77, 128.64, 124.60, 123.20, 115.84, 46.67, 40.31, 22.02; MS (CI) *m*/*z* 230 (M$^+$ + 1, 26). HRMS (CI) calcd for C$_{14}$H$_{16}$NO$_2$: 230.1181, found 230.1182.

5.2.16. *6-[2-(4-Hydroxy-phenyl)-butyl]-pyridin-3-ol (**28**)*

According to the general deprotection procedure, compound **26** (200 mg, 0.53 mmol) in 5.6 mL acetic acid was added to 1.46 mL hydriodic acid (57 wt.% in water) in 5.6 mL acetic acid and refluxed for 2 h. After reaction work up, purification by flash chromatography (80% ether/CH$_2$Cl$_2$) and additional purification by preparative TLC (80% ether/CH$_2$Cl$_2$), **28** was obtained as a white solid (74 mg, 57%, mp 83–88 °C). ^{1}H NMR (500 MHz, acetone-d_6) 8.08 (d, $J = 2.9$, 1H), 6.99–6.95 (m, 3H), 6.81 (d, $J = 8.4$, 1H), 6.70 (d, $J = 8.6$, 2H), 2.95–2.81 (m, 3H), 1.68–1.60 (m, 1H), 1.57–1.49 (m, 1H), 0.72 (t, $J = 7.4$, 3H); ^{13}C NMR (400 MHz, acetone-d_6) δ 156.30, 152.50, 152.41, 137.70, 136.49, 129.40, 124.54, 122.96, 115.71, 48.08, 45.14, 29.45, 12.35; MS (CI) *m*/*z* 244 (M$^+$ + 1, 13). HRMS (CI) calcd for C$_{15}$H$_{18}$NO$_2$: 244.1338, found 244.1341.

5.2.17. *Mixture of* cis- *and* trans-*5-(*tert*-butyl-dimethyl-silanyloxy)-2-[1-ethyl-2-(4-methoxy-phenyl)-propyl]-pyridine (**29**)*

According to the general alkylation procedure, **25** was lithiated by adding **25** (3.56 g, 9.97 mmol, in 3.8 mL THF) to a solution of LDA (5.00 mL of a 2 M solution in heptane/THF/ethylbenzene) in 3.8 mL THF. Adding ethyl bromide (1st addition: 750 µL, 10.05 mmol, in 3.8 mL THF, 2nd addition: 750 µL pure) to a stirred solution of lithiated **25** gave the mixture of diastereomers **29** as a yellow oil (*cis*-isomer: 1.19 g, 31%, *trans*-isomer: 470 mg, 12%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, acetone-d_6) δ 8.24 (d, $J = 2.9$, 0.4H), 8.11 (d, $J = 2.9$, 1H), 7.14 (d, $J = 8.8$, 0.8H), 7.08 (dd, $J = 2.9$, $J = 8.4$, 0.4H), 6.97 (d, $J = 8.4$, 0.4H), 6.87–6.82 (m, 3.8H) 6.64 (d, $J = 8.8$, 2H), 6.54 (d, $J = 8.4$, 1H), 3.80 (s, 1.2H), 3.71 (s, 3H), 3.05–2.93 (m, 1.4H), 2.71–2.60 (m, 1.4H), 1.93–1.85 (m, 1H), 1.80–1.71 (m, 1H), 1.53–1.47 (m, 0.4H), 1.44–1.38 (m, 0.4H), 1.31 (d, $J = 7.1$, 3H), 1.00 (s, 3.6H), 0.95–0.92 (m, 10.2H), 0.70 (t, $J = 7.4$, 3H), 0.54 (t, $J = 7.4$, 1.2H), 0.23 (s, 2.4H), 0.15 (d, $J = 1.7$, 6H). ^{13}C NMR (500 MHz, acetone-d_6) δ 157.79, 157.34, 156.46, 156.12, 150.12, 149.68, 141.69, 141.19, 138.77, 138.42, 128.44, 126.82, 126.41, 124.11, 124.05, 113.64, 113.05, 56.02, 55.89, 55.15, 55.01, 44.52, 44.03, 26.52, 25.58, 24.67, 21.12, 19.20, 18.17, 12.92, 12.15, −4.49, −4.45; MS (CI) *m*/*z* 386 (M$^+$ + 1, 100).

5.2.18. *Mixture of* cis- *and* trans-*5-(*tert*-butyl-dimethyl-silanyloxy)-2-[1-ethyl-2-(4-methoxy-phenyl)-butyl]-pyridine (**30**)*

According to the general alkylation procedure, **26** was lithiated by adding **26** (2.90 g, 7.82 mmol, in 3 mL THF) to a solution of LDA (4.69 mL of a 2 M solution in heptane/THF/ethylbenzene) in 3 mL THF. Adding ethyl bromide (1st addition: 700 µL, 9.37 mmol, in 3 mL THF, 2nd addition: 700 µL pure) to a stirred solution of lithiated **26** gave a 1:1 mixture of diastereomers **30** as a yellow oil (*cis*-isomer: 374 mg, 12%, *trans*-isomer: 374 mg, 12%) after reaction work up and purification (10% EtOAc/hexanes). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.24 (d, $J = 2.8$, 1H), 8.08 (d, $J = 2.8$, 1H), 7.11–7.06 (m, 3H), 6.96 (d, $J = 8.4$, 1H), 6.85 (d, $J = 8.6$, 2H), 6.81–6.76 (m, 3H), 6.62 (d, $J = 8.8$, 2H), 6.50 (d, $J = 8.4$, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 2.79–2.66 (m, 4H), 2.01–1.88 (m, 2H), 1.76–1.68 (m, 1H), 1.60–1.51 (m, 1H), 1.48–1.21 (m, 4H), 0.99 (s, 9H), 0.94 (s, 9H), 0.73–0.69 (m, 6H), 0.54–0.49 (m, 6H), 0.23 (s, 6H), 0.14 (d, $J = 1.7$, 6H); ^{13}C NMR (500 MHz, CDCl$_3$) δ 157.78, 157.32, 156.73, 156.16, 150.08, 149.61, 141.67, 141.09, 136.30, 135.75, 129.37, 129.23, 126.79, 126.34, 124.17, 123.98, 113.53, 112.90, 55.14, 55.04, 54.98, 54.74, 52.31, 51.97, 27.42, 26.54, 25.77, 25.59, 25.26, 18.18, 12.27, 12.10, −4.47, −4.56; MS (CI) *m*/*z* 400 (M$^+$ + 1, 100).

5.2.19. *Separated* cis- *and* trans-*6-[1-ethyl-2-(4-hydroxy-phenyl)-propyl]-pyridin-3-ol (**31a**/**31b**)*

According to the general deprotection procedure, the mixture of diastereomers **29** (780 mg; *cis*-isomer: 562 mg, 1.46 mmol, *trans*: 218 mg, 0.57 mmol) in 22 mL acetic acid was added to 5.5 mL hydriodic acid (57 wt.% in water) in 22 mL acetic acid and refluxed for 1.5 h. After reaction work up, the two diastereomers were purified by flash chromatography (75% ether/CH$_2$Cl$_2$) to give two fractions, each one enriched in one of the two isomers. Subsequently, preparative TLCs (50% ether/CH$_2$Cl$_2$) were performed with both fractions to give the pure *cis*-isomer **31a** (white solid, 371 mg, 66%, mp 90–95 °C) and *trans*-isomer **31b** (white solid, 135 mg, 62%, mp 80–85 °C). **31a**: ^{1}H NMR (500 MHz, acetone-d_6) 8.07 (d, $J = 2.9$, 1H), 6.89 (dd, $J = 2.8$, 8.5, 1H), 6.82 (d, $J = 8.5$, 2H), 6.66 (d, $J = 8.5$, 1H), 6.56 (d, $J = 8.5$, 2H), 3.03–2.97 (m, 1H), 2.73–2.69 (m, 1H), 1.86–1.71 (m, 2H), 1.24 (d, $J = 7.1$, 3H), 0.63 (t, $J = 7.4$, 3H); ^{13}C NMR (500 MHz, acetone-d_6) δ 155.93, 155.25, 152.19, 138.30, 137.56, 129.25, 125.00, 122.50, 115.35, 56.16, 44.61, 25.17, 19.70, 12.53; MS

(CI) m/z 258 ($M^+ + 1$, 55). HRMS (CI) calcd for $C_{16}H_{20}NO_2$: 258.1494, found 258.1497. **31b**: ^1H NMR (500 MHz, acetone-d_6) 8.23 (d, $J = 2.8$, 1H), 7.17 (dd, $J = 2.8$, 8.4, 1H), 7.09–7.06 (m, 3H), 6.78 (d, $J = 8.4$, 2H), 2.96–2.90 (m, 1H), 2.67–2.62 (m, 1H), 1.56–1.46 (m, 1H), 1.40–1.31 (m, 1H), 0.86 (d, $J = 7.1$, 3H), 0.49 (t, $J = 7.4$, 3H); ^{13}C NMR (500 MHz, acetone-d_6) δ 156.41, 155.49, 152.64, 138.52, 138.21, 129.19, 125.11, 122.92, 115.90, 56.35, 45.20, 27.23, 21.71, 12.43; MS (CI) m/z 258 ($M^+ + 1$, 100). HRMS (CI) calcd for $C_{16}H_{20}NO_2$: 258.1494, found 258.1486.

5.2.20. Separated *cis*- and *trans*-6-[1-ethyl-2-(4-hydroxy-phenyl)-butyl]-pyridin-3-ol (*32a*/*32b*)

According to the general deprotection procedure, the mixture of diastereomers **30** (374 mg (187 mg of each isomer), 0.94 mmol) in 20 mL acetic acid was added to 2.6 mL hydriodic acid (57 wt.% in water) in 10 mL acetic acid and was refluxed for 1.5 h. After reaction work up, the two diastereomers were purified by flash chromatography (75% ether/CH_2Cl_2) to give two fractions, each one enriched in one of the two isomers. Subsequently, preparative TLCs (50% ether/CH_2Cl_2) were performed with both fractions to give the pure *cis*-isomer **32a** (white solid, 91 mg, 72%, mp 83–88 °C) and *trans*-isomer **32b** (white solid, 95 mg, 75%, mp 83–88 °C). **32a**: ^1H NMR (500 MHz, acetone-d_6) 8.05 (d, $J = 2.8$, 1H), 6.87 (dd, $J = 2.8$, 8.4, 1H), 6.76 (d, $J = 8.6$, 2H), 6.62 (d, $J = 8.4$, 1H), 6.57 (d, $J = 8.6$, 2H), 2.81–2.73 (m, 2H), 1.97–1.82 (m, 2H), 1.78–1.69 (m, 1H), 1.58–1.49 (m, 1H), 0.69–0.63 (m, 6H); ^{13}C NMR (500 MHz, acetone-d_6) δ 155.94, 155.27, 152.06, 137.54, 135.54, 130.24, 125.02, 122.29, 115.24, 54.92, 52.52, 26.30, 25.70, 12.55; MS (CI) m/z 272 ($M^+ + 1$, 66). HRMS (CI) calcd for $C_{17}H_{22}NO_2$: 272.1651, found 272.1650. **32b**: ^1H NMR (500 MHz, acetone-d_6) 8.21 (d, $J = 2.9$, 1H), 7.14 (dd, $J = 2.9$, 8.4, 1H), 7.06–7.04 (m, 3H), 6.79 (d, $J = 8.4$, 2H), 2.71–2.67 (m, 2H), 1.50–1.41 (m, 1H), 1.35–1.16 (m, 3H), 0.50–0.45 (m, 6H); ^{13}C NMR (500 MHz, acetone-d_6) δ 156.48, 155.77, 152.64, 138.23, 135.97, 130.02, 125.07, 122.98, 115.89, 54.45, 53.01, 28.14, 27.30, 12.44, 12.36; MS (CI) m/z 272 ($M^+ + 1$, 100). HRMS (CI) calcd for $C_{17}H_{22}NO_2$: 272.1651, found 272.1659.

5.3. Estrogen receptor binding affinity assays

Relative binding affinities were determined by a competitive radiometric binding assay as previously described [37,38], using 10 nM [^3H]estradiol as tracer (GE Healthcare, Piscataway, NJ), and purified full-length human ERα and ERβ (PanVera/InVitrogen, Carlsbad, CA). Incubations were for 18–24 h at 0 °C, then the receptor–ligand complexes were adsorbed onto hydroxyapatite (BioRad, Hercules, CA) and unbound ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values, with the RBA of estradiol set to 100. The values given are the average ± range or SD of two or more independent determinations. Estradiol binds to ERα with a K_d of 0.2 nM and to ERβ with a K_d of 0.5 nM.

5.4. Cell culture and transient transfections

Human endometrial cancer (HEC-1) cells were maintained in Minimum Essential Medium (MEM) plus phenol-red supplemented with 5% calf serum and were changed to phenol-red-free Improved MEM and 5% charcoal dextran-treated calf serum (CDCS) for 3–4 days before use in experiments. Transfection assays were performed in 24-well plates using a mixture of 0.35 mL of serum-free improved MEM medium and 0.15 mL of Hank's balanced salt solution containing 5 μg of lipofectin (Life Technologies, Inc., Gaithersburg, MD), 20 μg of transferrin (Sigma, St. Louis, MO), 0.2 μg of pCMV β-galactosidase as internal control, 0.5 μg of 2ERE-pS2-Luc, and 50 ng of ER expression vector per well. The cells were incubated at 37 °C in a 5% CO_2-containing incubator for 6 h. The medium was then replaced with fresh Improved MEM supplemented with 5% CDCS plus the desired concentrations of ligands. Cells were harvested 24 h later. Luciferase activity and β-galactosidase activity were assayed as described [41].

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