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Ring nitrogen-substituted non-steroidal estrogens: pyridine and pyrimidine analogs of the phenol in deoxyhexestrol experience resonance constraints on preferred ligand conformation

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Abstract—To develop compounds selective for estrogen receptor beta (ERβ), we substituted hydroxypyridine and pyrimidine heteroaryl groups for the characteristic phenol ring of nonsteroidal estrogens. The unexpectedly low affinity showed by some of these compounds is ascribed, in part, to a resonance-enforced conformational constraint that prevents their optimal accommodation in the ER ligand binding pocket.

The estrogen receptor (ER) is a member of the nuclear hormone receptors superfamily and plays an important role in the development and function of female reproductive system and in the maintenance of bone mineral density and cardiovascular health.[1–3] Despite these positive effects, the activation of ER in some tissues such as breast and uterus can increase the risk of cancer in these sites.[4] Consequently, the development of compounds that can maintain the benefits of estrogen while avoiding the risks is a major current challenge. Such compounds are termed selective estrogen receptor modulators or SERMs.[5]

The recent discovery of a second estrogen receptor[6,7] (ERβ), with tissue distribution and transcriptional properties different from ERα, opens new possibilities for developing tissue and cell-selective estrogens based on preferential binding or activation of these two ERs. ERα and ERβ have a conserved DNA-binding domain, and although the amino acid identity of their ligand-binding domains is only 59%, their ligand binding pockets (LBPs) are almost identical, the differences being a somewhat smaller internal volume for ERβ and the substitution of just two amino acids: Met421 in ERα corresponding to Ile in ERβ, and Leu384 in ERα to Met in ERβ.[8] The high sequence similarity of the LBPs presents a challenge for the development of ER subtype-selective compounds. There are a number of estrogens with good selectivity for ERα,[9,10] but fewer ERβ-selective compounds are known. The isoflavone phytoestrogen genistein (**1**) shows 20-fold selectivity toward ERβ,[11] and similar selectivity is reported for some aryl benzothiophene derivatives;[12] we reported a 70-fold selectivity for compound **2** (Fig. 1).[13]

Recently, other more polar heterocyclic core systems—benzothiazoles, benzimidazoles, and benzoxazoles—have been described as ERβ-selective agents;[14–16] compound **3**, in particular, has a remarkable 203-fold selectivity. Notable in these ligands is a relatively narrow structural profile, with a core system enriched

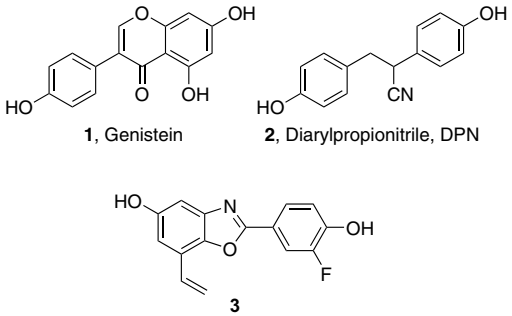

Figure 1. Some compounds selective for ERβ.

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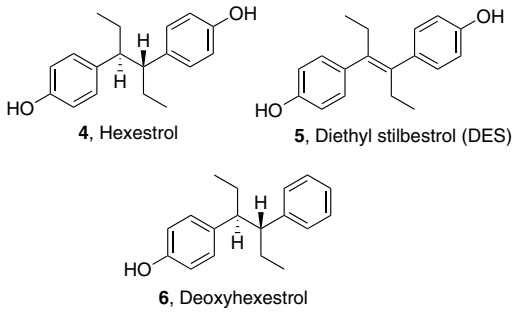


Figure 2. Reference compounds for pyridine and pyrimidine analogs.

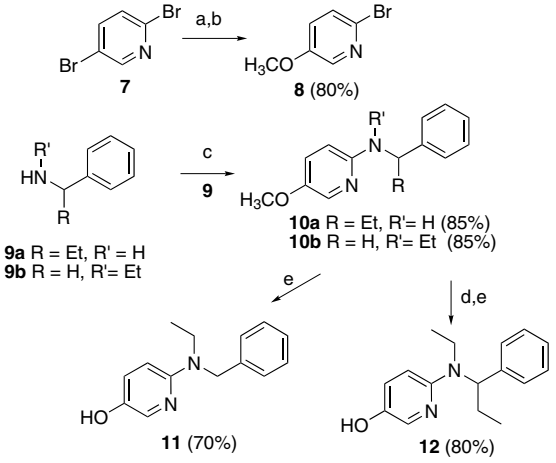
in heteroatoms, characteristics that appear favor ERβ-selective binding (though do not appear to be essential).[17] Recently, deoxyhexestrol (Fig. 2), a compound we first examined long before the discovery of ERβ, was tested again for its binding to both ERs; it had good affinity, especially for ERβ, being in this regard more preferential than its congeners hexestrol and diethylstilbestrol (DES) (Table 1).

To develop other ERβ-selective ligands of novel structure, we considered analogs of deoxyhexestrol in which the phenolic ring would be replaced with either 5-hydroxypyridine or 5-hydroxypyrimidine units (see **12** and **17**, both of which also contain a 2-amino group). In this manner, we would be substituting heterocyclic analogs for the phenol, the most characteristic functionality of estrogens, a group that is present in almost all nonsteroidal estrogens and is known to be a dominant feature in the binding of estrogens to the ER. By basing the design of these new ligands on deoxyhexestrol, we ensured that there would not be another phenol in the molecule. Thus, we presumed that the hydroxypyridine or hydroxypyrimidine unit would, of necessity, replace the phenol in binding to the ERs. Also, because these two heteroaryl moieties have somewhat lower hydrophobicities than phenols, yet (particularly with the 2-amino group) are not excessively basic (see below), we thought they might be well accommodated within the ER LBPs, perhaps preferentially within what appears to be the more polar-tolerant ERβ LBP. Some aryl systems containing one or more nitrogen atoms have been reported as ER ligands,[18,19] and our selection of the pyridine and pyrimidine systems was encouraged by these precedents, as well as ease of synthesis considerations. Herein, we present the synthesis of six new pyridine- and pyrimidine-based nonsteroidal ER ligands (compounds **11**, **12**, **16**, and **17**, **21a**, **21b**) and an analysis of their ER binding affinities.

Table 1. ERα and ERβ binding affinity and selectivity, and Clog *P* of compounds in this study and related compounds for comparison

Compound	Relative binding affinity (RBA)[a]		β/α Ratio	Clog *P*
	ERα	ERβ		
Hexestrol (**4a**)	280	700	2.5	5.11
Isobutestrol (**4b**)	2.2	40	18	4.18
DES (**5**)	430	280	0.6	4.95
Deoxyhexestrol (**6**)	18	73	4	5.78
11	0.019	0.026	1.3	3.94
12	0.030	0.077	2.5	4.78
16	0.007	0.004	0.5	3.51
17	0.007	0.014	2.0	4.35
21a	0.081	0.684	8.4	5.23
21b	0.010	0.023	2.3	5.23

[a] RBA values are determined by a competitive binding assay, using [^{3}H]estradiol as tracer, according to a published method.[22] We have found that this assay consistently gives RBA values with CVs of ⩽0.3.

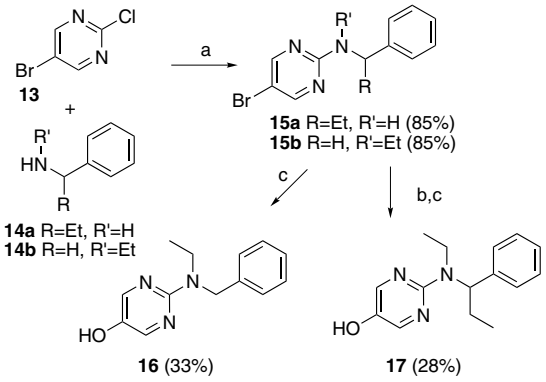


Scheme 1. Reagents and conditions: (a) BuLi, B(O*i*Pr)$_3$, H_2O_2, ether −78 °C; (b) NaH, MeI, DMF, rt; (c) $Pd_2(dba)_3$ (2 mol%), NaO*t*Bu, (±)-Binap (4 mol%), toluene 70 °C (for **10a**) $Pd_2(dba)_3$ (1.5 mol%), NaO*t*Bu, (−)Ppfa (4.5 mol%), toluene, 70 °C (for **10b**); (d) NaH, EtI, DMF, reflux; (e) NaH, EtSH, DMF, reflux.

The efficient synthesis of pyrimidine derivatives **11** and **12** was accomplished by the procedure shown in Scheme 1. It was possible to synthesize 2-bromopyridinyl-5-boronic ester from 2,5-dibromopyridine (**7**) through a selective C-5 lithiation,[20] followed by quenching with triisopropyl borate. Oxidation of the resulting boronic ester with hydrogen peroxide[21] was followed by protection of a hydroxyl group as the methyl ether, giving compound **8**. Palladium-mediated coupling of this bromopyridine with the substituted benzyl amines **9a**,**b** gave aminopyridine products **10a**,**b**, but the best yields were obtained with different catalytic systems: $Pd_2(dba)_3$ and (±)Binap for **10a** and $Pd_2(dba)_3$ and (−)Ppfa for **10b**.

Compound **10a** was then alkylated and subsequently deprotected to give derivative **12**. Compound **11** was obtained from derivative **10b** after a facile deprotection. Derivatives **10a**,**b** were both deprotected under basic conditions, instead of the usual acid ones, because compounds **11** and **12** are not stable in acid.

The synthesis of pyrimidine analogs **16**,**17** shown in Scheme 2, was accomplished in good yield, in 2–3 steps. In a sealed tube, 2-choloro-5-bromo-pyrimidine (**13**) and benzyl amines **14a**,**b** were heated at 150 °C for 40 min, in



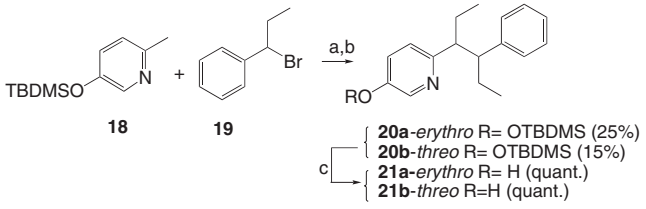
Scheme 2. Reagents and conditions: (a) *i*PrOH, diisopropylethylamine, 150 °C, sealed tube; (b) NaH, EtI, DMF, rt; (c) BuLi, $B(OiPr)_3$, H_2O_2, −78 °C.

the presence of iPr_2NEt, to give the aminopyrimidines **15a**,**b**. Derivative **15a** was then alkylated, and the bromine substituent converted to the OH group as done previously for compound **8**, giving compound **17**. Compound **16** was obtained from derivative **15b** in a similar manner.

To better compare the activity of the previous derivatives with deoxyhexestrol, we also planned to synthesize pyridine analogs in which the benzylic nitrogen is substituted with a carbon, and compounds in which a *p*-OH-aniline replaces the phenol. The synthesis of the pyridine derivatives (Scheme 3) was accomplished treating picoline **18**, with LDA in the presence of 1-bromo-propylbenzene **19**. The compound obtained was then alkylated and deprotected to give diasteroisomers **21a**, **21b**. Unfortunately, we were unable to prepare the corresponding aniline analogs, because these derivatives proved to be very unstable after deprotection.

Because nonsteroidal bisphenols are generally of higher affinity ligands than monophenols, we also attempted to prepare a series of derivatives in which the phenyl group was replaced by a 4-hydroxyphenyl ring. However, these compounds also proved to be unstable after deprotection, presumably experiencing a facile 1,6-elimination of the benzylic amine via a quinonemethide intermediate.

The binding affinity of the six compounds prepared above, for human ERα and ER was determined using



Scheme 3. Reagents and conditions: (a) LDA, −78 °C, THF; (b) LDA, EtBr, −78 °C, THF; (c) TBAF, 0 °C, THF.

a competitive radiometric assay; affinities are expressed relative to that of estradiol (100%) to give relative binding affinity (RBA) values (Table 1).[22] The binding affinity of all of the nitrogen-substituted analogs (**11**, **12**, **16**, **17**, **21a**,**b**) was much lower than that of the reference compounds (**4a**,**b**, **5**, **6**). The *erythro* derivative **21a** is the highest binder and most ERβ-selective compound of the new compounds, the other compounds binding considerably less well than **21a**.

It is of note that the aminopyridine and aminopyrimidine systems (**11**, **12** and **16**, **17**) are not very basic (calculated pK_a values are 6.7 and 3.9 for the two series; ACD Labs), nor do any of the ligands studied have an overall lipophilicity (estimated from Clog *P*) that places them far out of the range of other high affinity ER ligands; yet, they are still not effective analogs of the carbocyclic systems, such as deoxyhexestrol (**6**). Nevertheless, some trends in the binding data should be noted: (1) RBA values increase when the central atoms that link the two rings are both alkylated (compare **11** vs **12** and **16** vs **17**, Table 1), consistent with the higher affinity of the doubly alkylated all-carbon analogs, hexestrol (**4a**) versus isobutestrol (**4b**).[23] From X-ray crystal structures, it is known that these two ethyl groups nicely fill preformed pockets in the ER that are above position C-11β and below position C-7α of estradiol.[24] (2) Of the two series, the pyridine derivative **21a** binds 2–10 times better than compound **12**, and derivatives **11**, **12** bind 2–5 times better than the pyrimidine analogs **16**, **17**, consistent with the higher Clog *P* values of the former system (Table 1). (3) The *erythro* isomer **21a** binds better than *threo* isomer **21b**, consistent with the behavior reported for their phenol analogs.[23]

One possible reason for the low affinity shown by derivatives **11**, **12**, **16**, and **17** is that they lack a second hydroxyl group, but comparing the RBA data of these derivatives with that of the reference monophenol **6**, it seems that they are in some other general ways poorly suited for the ER LBPs. One possible factor could arise from resonance overlap between the heterocycle and the 2-amino substituent: the need for π overlap is expected to induce sp^2 hybridization in the benzylic nitrogen whose substituents should consequently be constrained to be in the same plane as the heterocycle (angle θ in Fig. 3). This constraint could also have a secondary effect on the conformation of the amine substituents, with the result that the overall conformation of the heterocyclic analog of deoxyhexestrol might be such that it cannot be optimally accommodated within the ER LBP. To investigate this possibility, we used quantum mechanical calculations (6-31G*/B3LYP) to compare minimum energy conformations of hexestrol, DES, and derivatives **12**, **17** (Fig. 3).

It is of note that the minimum energy conformation of DES matches quite closely the conformation it was shown to adopt in the crystal structure with ERα: the angle θ shown in Figure 3 is close to 90°, as in the experimental structure.[24] The computed structure of hexestrol is similar to that of DES, with $\theta = 86°$ and the ethyl substituent equally extended. By contrast, because of the

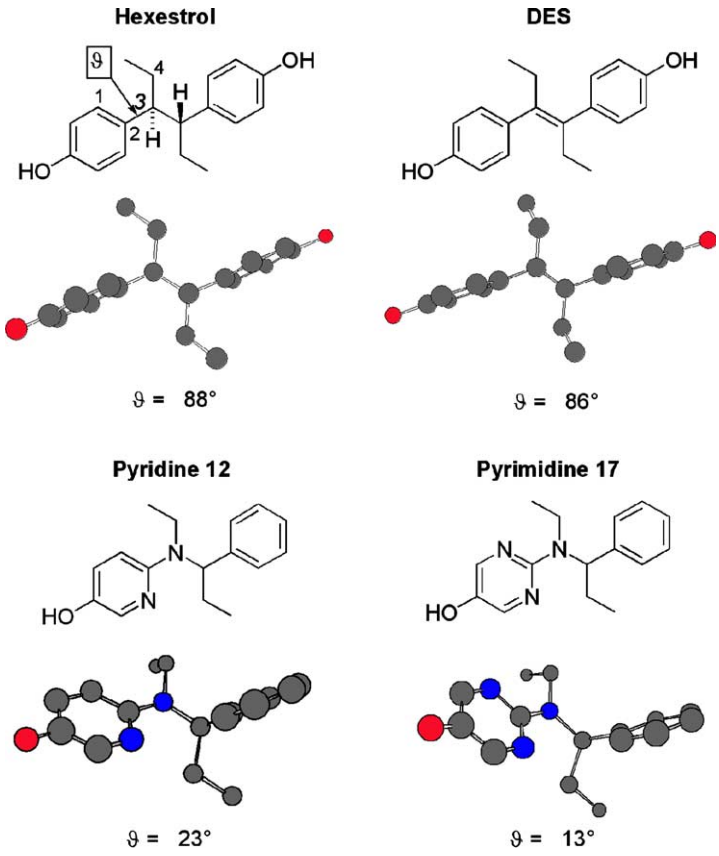


Figure 3. Dihedral angle θ is defined by atoms 1–4.

stronger resonance-induced planarity, the heteroarenes are constrained to much lower dihedral angles, between 13° and 23° with respect to the central bond (Fig. 3).

DES (and by analogy, hexestrol) fits very well to the ER: one phenol fits in the narrow A-ring binding pocket just does as the A-ring of estradiol, and because of the 86° ring/3-hexene backbone torsional angle, the two ethyl groups can nicely fill the major 7α, 11β subpockets.[24] By contrast, because of the more pronounced amine-heterocycle resonance, the pyridine and pyrimidine analogs of deoxyhexestrol, **12** and **17**, appear to be forced to adopt a conformation in which the backbone is almost coplanar with the hydroxy-containing heteroarene (presumed to be the A-ring phenol mimic), with the result that the two ethyl groups are not well disposed to fill the 7α, 11β subpockets. Therefore, we believe that the low ER binding affinity of the 2-aminopyridine and pyrimidine analogs for ER arises—in large part—from a nonoptimal conformation, strongly enforced by resonance, which is poorly suited for binding to the receptor, although other factors (unsatisfied polar sites on heteroaryl groups or alterations in hydrogen bonding) might also be playing a role. Although this argument alone cannot account for the low affinity of the pyridine analog **21a** (which has a calculated θ value of 79°, similar to hexestrol), it is of note that this system is considerably more basic (calculated pK_a of 6.0) and its affinity is considerably higher than that of the corresponding 2-amino analog **12** on both ERs.

In summary, we have investigated the substitution of the phenolic ring, a characteristic structural motif in estrogen receptor ligands, with a 5-hydroxypyridine or a 5-hydroxypyrimidine, to investigate the extent of heteroatom tolerance of the ER subtypes. Although derivative **21a** is more selective then deoxyhexestrol (**6**) toward ERβ, with an RBA close to 1% for this receptor, all other compounds prepared, however, showed surprisingly weak affinity for both receptors. Nevertheless, the low binding affinity of these last pyridine and pyrimidine non-steroidal estrogens (**12** and **17**) is instructive because it shows that introduction of nitrogen heteroatoms within the flexible structure of a high affinity all-carbon ligand, deoxyhexestrol, can dramatically reduce binding affinity, even without altering the overall hydrophobicity of the ligand, most likely because of resonance-induced conformational constraints. This information will prove useful in directing our continued efforts to prepare novel ER ligands with high affinity and subtype selectivity.

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References and notes

1. Davidson, N. E. *N. Engl. J. Med.* **1995**, *352*, 1638.
2. Barrett-Conner, E.; Bush, T. L. *J. Am. Med. Assoc.* **1991**, *265*, 1861.
3. Yaffe, K.; Sawaya, G.; Lieberbung, I.; Grady, D. *J. Am. Med. Assoc.* **1998**, *279*, 688.
4. Beresford, S. A.; Weiss, N. S.; Voigt, L. F.; McKnight, B. *Lancet* **1997**, *349*, 458.
5. Dutertre, M.; Smith, C. L. *J. Pharm. Exp. Ther.* **2000**, *295*, 431.
6. Mosselman, S.; Polman, J.; Dijekema, R. *FEBS Lett.* **1996**, *392*, 49.
7. Kuiper, G. G. J. M.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J.-Å. *PNAS* **1996**, *93*, 5925.
8. Pinke, A. C. W.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A.-G.; Engström, O.; Ljunggren, J.; Gustafsson, J.-Å.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608.
9. Sun, J.; Meyers, M. J.; Fink, B. E.; Rajendran, R.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **1999**, *140*, 800.
10. Mortensen, D. S.; Rodriguez, A. L.; Carlson, K. E.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 3838.
11. Kuiper, G. G. J. M.; Carlsson, B.; Grandien, J.; Enmark, E.; Häggblad, J.; Nilsson, S.; Gustafsson, J.-Å. *Endocrinology* **1997**, *138*, 863.
12. Schopfer, U.; Schoeffter, P.; Bischoff, S. F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. *J. Med. Chem.* **2002**, *45*, 1399.
13. Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230.
14. AstraZeneca A. B. WO Patent 02/051821-A1, 2002.
15. AstraZeneca A. B. WO Patent 02/46168-A1, 2002.
16. Wyeth, WO Patent 03/050095-A1, 2003.
17. Edsall, R. J.; Harris, H. A.; Manas, E. R.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2003**, *11*, 3457.
18. Henke, B. R.; Drewry, D. H.; Jones, S. A.; Stewart, E. L.; Weaver, S. L.; Wiethe, R. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1939.
19. Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.; Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin, J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisley, G. B.; Lambert, M. H. *J. Med. Chem.* **2002**, *45*, 5492.
20. Bouillon, A.; Lancelot, J.-C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 2885.
21. Krow, G. R.; Xiao, Y.; Cannon, K.; Swan, S. A.; Nickel, A. *Synth. Commun.* **2000**, *30*(22), 4093.
22. Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *Biochemistry* **1997**, 14897.
23. Kilbourn, M. R.; Arduengo, A. J.; Park, J. T.; Katzenellenbogen, J. A. *Mol. Pharmacol.* **1981**, *19*, 388.
24. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.