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Pyrazolo[1,5-*a*]pyrimidines as estrogen receptor ligands: defining the orientation of a novel heterocyclic core

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Abstract—We have examined the pyrazolo[1,5-*a*]pyrimidine scaffold as a novel core structure for estrogen receptor ligands. Attachment of various substituents has helped to define the orientation of this heterocycle in the ligand-binding pocket as one in which a pendant phenol rather than the hydroxylpyrimidine serves as a mimic of the A-ring of estradiol.

The estrogen receptor (ER) is a nuclear hormone receptor of pharmaceutical interest as a target for the treatment of osteoporosis, breast cancer, and other endocrine disorders.[1] Although its natural ligand is the steroid 17β-estradiol (E_2), the ER is capable of binding a wide variety of steroidal and nonsteroidal ligands.[2] Many ER ligands are built upon a heterocyclic core as a scaffold onto which are attached additional substituents. Among these cores are 5,6-fused heterocycles (Fig. 1A), such as the benzothiophenes[3] and indoles.[4] Both of these systems, as well as others, incorporate a hydroxyl group into the six-membered ring that serves as a mimic of the A-ring phenol of estradiol. As part of our larger effort to diversify the core structures of ER ligands,[5–7] we considered the pyrazolo[1,5-*a*]pyrimidine system (Fig. 1B): This structure is commensurate with other 5,6-fused heterocycles, is easily synthesized, and can readily be adorned with substituents. The versatility of the core has allowed it to be studied as a scaffold in a wide variety of pharmaceutical targets. Thus, the pyrazolo[1,5-*a*]pyrimidine scaffold has recently be examined as a CRF antagonist[8] and COX-2 inhibitor.[9]

In our initial consideration of the pyrazolo[1,5-*a*]pyrimidine core as an ER ligand building block, we incorporated a hydroxyl at the 6-position, in a manner similar to the benzothiophene and indole systems, and we appended aromatic rings at the 2- and 3-positions, which

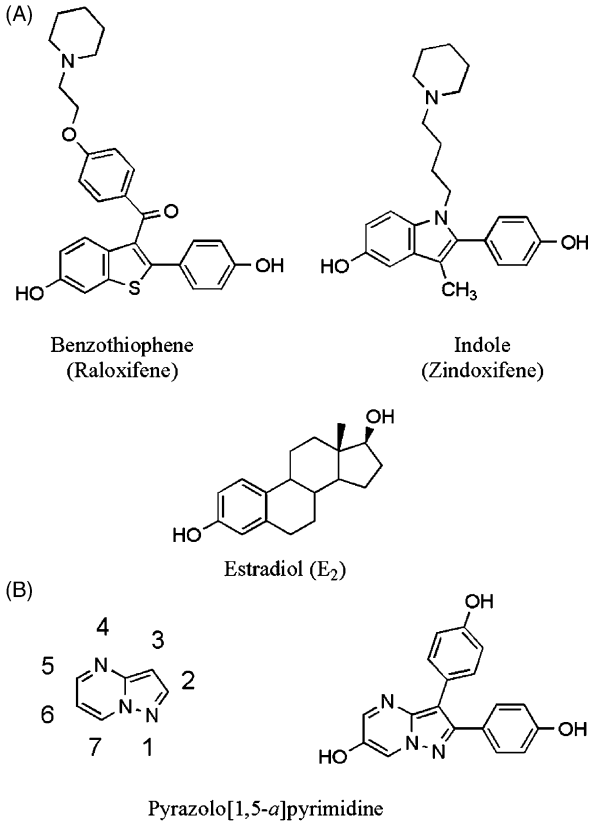

Figure 1. (A) Benzothiophene and indole heterocycles, (B) pyrazolo[1,5-*a*]pyrimidine core and numbering system. 6-Hydroxypyrazolo[1,5-*a*]pyrimidines a potential heterocyclic estrogen receptor ligand.

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are typically sites for aryl or alkyl group substitution on related systems. By incorporating a hydroxyl group into the 6-position of the core scaffold, we believed that the hydroxylpyrimidine portion would be poised to act as a mimic of the aromatic A-ring of estradiol. The aromatic ring substituents in such ligands can either be phenyl or 4-hydroxyphenyl groups, the latter depicted in Figure 1B.

Although we envisioned that the 6-hydroxyl group would act as the A-ring mimic of estradiol and hydrogen bond with Glu353 and Arg394 in the ligand-binding pocket of ERα, when multiple hydroxyl groups are present (i.e., with 4-hydroxyphenyl substituents), the possibility exists that one of the other rings could act as the A-ring mimic. To examine whether the 6-hydroxypyrimidine system could act as the A-ring mimic of E_2, and whether the compounds would fit into the binding pocket of the estrogen receptor, we docked the trihydroxy pyrazolopyrimidine system into the crystal structure of ERα ligand-binding domain complex with E_2 (1ERE) (Fig. 2).[10]

Using an approach we have reported previously,[6] we overlaid the 6-hydroxyl substituent with the hydroxyl of E_2 and allowed the structure to minimize in the receptor after E_2 was deleted from the pocket. In this manner, we found that the binding pocket would accommodate this system, with the 6-hydroxy-pyrazolo[1,5-*a*]pyrimidine acting as an A-ring mimic of the phenol of E_2. In addition to forming the desired hydrogen bonds with Glu353 and Arg394, the modeling also indicated potential hydrogen bonding between Thr347 and hydroxyl of the phenyl at the 2-position. In further modeling studies, we examined the possibilities of either of the additional 4-hydroxyphenyl rings acting as the A-ring mimic of E_2 (results not shown). We found that 2,3-bis-(4-hydroxyphenyl)-pyrazolo[1,5-*a*]pyrimidines could also be accommodated in an alternate orientation, with the pendant C(2)-phenol in the A-ring binding site (not shown). Considering these alternative possibilities and recognizing that molecular modeling would not provide a definitive means for selecting among them, we set about synthesizing a set of molecules to explore which binding orientation to the ER was most likely to occur, that is, which ring would be the mimic of the A-ring of estradiol. Once the core orientation was established, we felt that we would be in a better position to explore additional systems to optimize ligand binding.

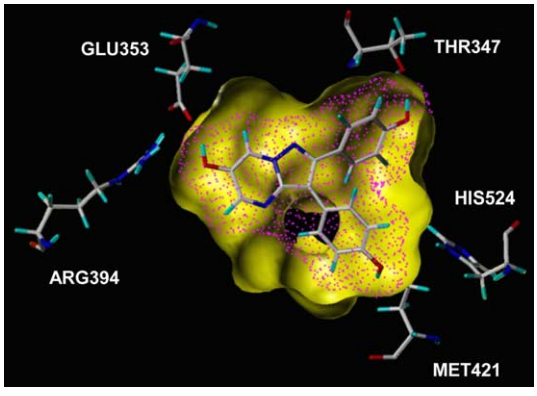
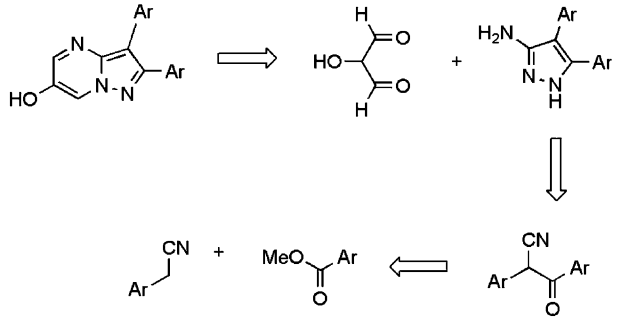


Figure 2. Docking of 2,3-bis-(4-hydroxyphenyl)-6-hydroxypyrazolo-[1,5-*a*]pyrimidine into the ERα binding pocket (1ERE), using SYBYL V. 6.6. Shown is the model with the 6-hydroxyl group mimicking the phenol of estradiol.

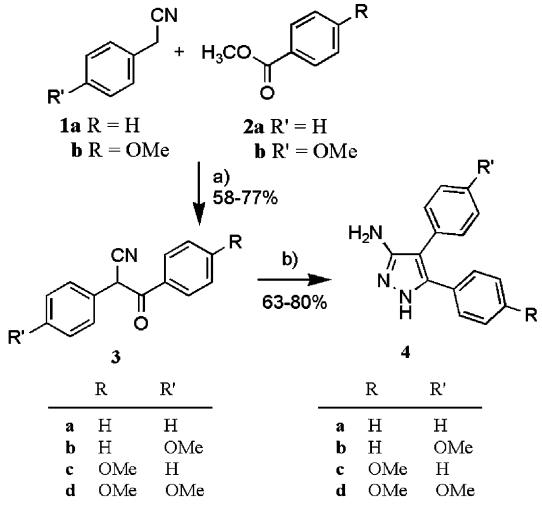
Pyrazolo[1,5-*a*]pyrimidines have historically been synthesized by a condensation of a dicarbonyl compound with a 3-amino-pyrazole under acidic conditions.[11,12] For the systems of interest to us, this involves the condensation of a substituted 1,3-propanedial and a 3-amino-4,5-diaryl-pyrazole (Scheme 1). The 3-aminopyrazole derivatives can be obtained in two steps, by Claisen condensation of a phenylacetonitrile with a methyl benzoate, followed by cyclization with hydrazine. The desired pyrazolo[1,5-*a*]pyrimidine can be then prepared in a few steps.

The synthesis of the desired 3-aminopyrazoles is illustrated in Scheme 2. A Claisen condensation between a phenyl acetonitrile **1a**–**b** and a methyl benzoate **2a**–**b** produces the 3-ketopropionitriles **3a**–**d** in good yields.[13] Condensation of compounds **3a**–**d** with hydrazine in the presence of acid produces the desired 3-aminopyrazoles **4a**–**d**.

The required 2-hydroxy-1,3-propanedial is a highly functionalized system that is not likely to be stable.



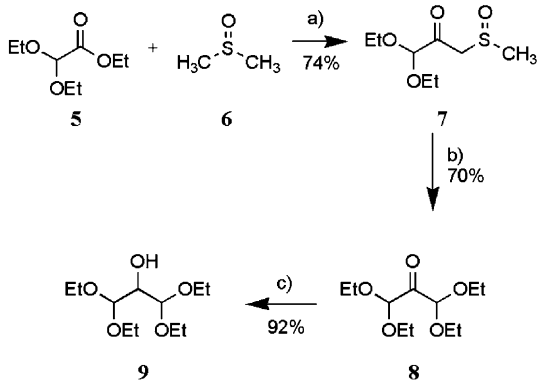
Scheme 1.



	R	R'
a	H	H
b	H	OMe
c	OMe	H
d	OMe	OMe

	R	R'
a	H	H
b	H	OMe
c	OMe	H
d	OMe	OMe

Scheme 2. Reagents and conditions: (a) THF, NaH, 65 °C, 2 days; (b) H_2NNH_2, EtOH, HCl, 80 °C, 8 h.



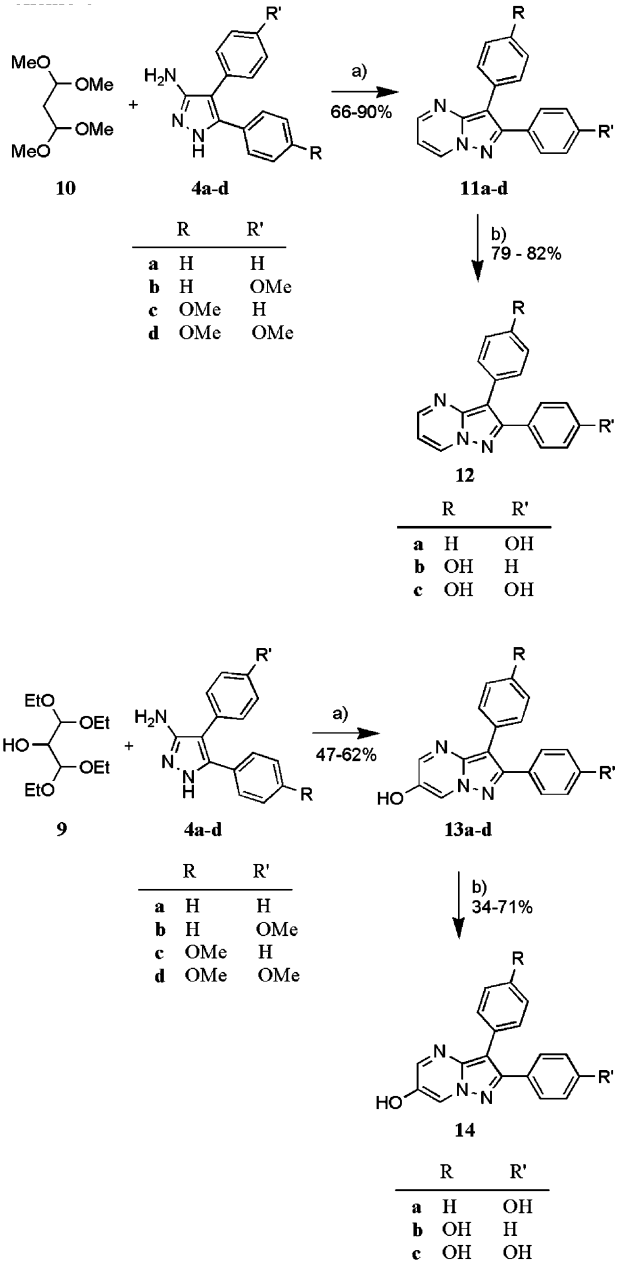
Scheme 3. Reagents and conditions: (a) NaH, rt, 6 h; (b) EtOH, $BF_3{\cdot}OEt_2$, Hg(II)O; (c) EtOH, $LiBH_4$, 7 h.

Therefore, we prepared it as the tetraalkoxy-bisacetal. Balsevich[14] prepared the 2-keto-1,1,3,3-tetraethoxy-propane **8** (Scheme 3) by a procedure that involves coupling of dimethylsulfoxide **6** with ester **5** to generate the β-ketosulfoxide **7**. Pummerer rearrangement of **7** with mercuric oxide yields ketone **8**. Baganz[15] prepared **8** via an alternative method; however, they described the reduction of **8** with lithium borohydride to give **9**, the 1,3-propanedial derivative we desired. The preparation of **9** allows for the synthesis of 6-hydroxypyrazolo[1,5-*a*]pyrimidines.

To obtain a more complete picture of the potential of the core to act as an estrogen receptor ligand and to study the cyclization conditions, we purchased 1,1,3,3-tetramethoxypropane (**10**), a compound that lacks the hydroxyl group needed to produce the 6-hydroxyl in the pyrazolo[1,5-*a*]pyrimidines, and we also reacted it with compounds **4a**–**d** (Scheme 4). For the in situ conversion of the bis-acetal to the reactive dialdehyde, a solvent sufficiently polar to dissolve the 3-aminopyrazoles and acid was required. Thus, in acetic acid the reaction reached completion in just a few hours, providing compounds **11a**–**d**. The methyl ethers of **11b**–**d** were cleaved with $BF_3{\cdot}SMe_2$ to reveal the free hydroxyls in compounds **12a**–**c**. The lack of the 6-hydroxyl group in these systems allowed us to study the possibility of the pendant hydroxyl as the A-ring mimic of estradiol.

Having established conditions for pyrazolo[1,5-*a*]pyrimidine formation and phenol deprotection, we prepared the desired analogs containing the 6-hydroxyl group (Scheme 4) by reaction of compounds **4a**–**d** with **9** to yield compounds **13a**–**d**. Deprotection of **13b**–**d** as above provided the desired products **14a**–**c**, which incorporated a hydroxyl group at the 6-position.

The two series produced a total of eight compounds whose binding for both ERα and ERβ was determined using a competitive radiometric assay.[16] The affinity of the compounds are expressed as a percent relative to estradiol, which is normalized to 100%. These relative binding affinity (RBA) values are given in Table 1. Although the binding affinities are low, the structure–affinity relationships are informative of the key issue of ligand core orientation.



Scheme 4. Reagents and conditions: (a) HOAc, 110 °C, 3 h; (b) CH_2Cl_2, $BF_3{\cdot}SMe_2$, 16–20 h.

The first compound to notice is **11a**; it has no hydroxyl groups and shows no binding to either ERα or ERβ. This indicates that the pyrazolo[1,5-*a*]pyrimidine core simply acts as a scaffold for the substituents to hydrogen bond with the ER and does not by itself interact in an effective manner directly with the receptor. Compound **13a**, which only contains the 6-hydroxyl for hydrogen bonding, shows at most minimal binding to the ER. To best understand the remaining data, compounds **12a**–**c** should be compared to **14a**–**c**, respectively. From this comparison, it is evident that with one exception, compounds containing a 6-hydroxyl substituent (**14a**–**c**) consistently show significantly *lower* RBA values than the corresponding systems without the 6-hydroxyl substituent (**12a**–**c**), not at all what we had originally sought. Also of note is that all compounds having

Table 1. ERα and ERβ relative binding affinities[a]

Compound	ERα	ERβ
11a	—	—
12a	0.010	0.050
12b	0.007	0.023
12c	0.004	0.100
13a	—	0.005
14a	0.003	0.010
14b	—	0.006
14c	0.004	0.034

[a] Relative binding affinities (RBA) reported as a percent of E_2 activity where $E_2 = 100\%$. These values are the average of replicate experiments, where the range has a CV below 0.3. Where values are not given, they are below detection, <0.003. The K_d of estradiol is 0.2 nM (ERα) and 0.5 nM (ERβ).

measurable RBA values are selective for ERβ. Compound **12c**, which had the highest ERβ binding, also showed the greatest selectivity (25-fold). Compound **14c**, of the original design, did show good ERβ selectivity, but had only a third the binding affinity of **12c**.

Based on the consistently higher affinity of the pyrazolo[1,5-*a*]pyrimidine systems *lacking* the 6-hydroxyl group, we believe it unlikely that this heterocyclic system binds with the 6-hydroxyl group mimicking the A-ring phenol of estradiol. Thus, in work to be described elsewhere, we used the highest affinity analog lacking the 6-hydroxyl group (**12c**) as a lead to explore how effectively systems that use the pendant 4-hydroxyphenyl groups as the A-ring mimic of estradiol can be developed to bind to the ERs.[17] In exploring these other analogs, we chose those that include alkyl groups at the 5- and 7-positions (Fig. 3). These alkyl groups could readily be incorporated into our synthetic scheme as substituents on the 1,3-dicarbonyl unit used to complete the formation of the pyrimidine unit, and they add hydrophobic bulk to fill space within the ligand-binding pocket. As described elsewhere, some of these redesigned pyrazolo[1,5-*a*]pyrimidine systems show significantly improved ER binding affinities, results which can be rationalized by molecular modeling which places the C-2 (4-hydroxyphenyl) group in the A-ring binding pocket. The new ER analogs also display interesting patterns of selectivity for ERα versus ERβ in terms of binding affinity and transcription potency and efficacy.[17]

In summary, we have explored a new heterocyclic system, pyrazolo[1,5-*a*]pyrimidine, as a core scaffold for developing novel ER ligands. This synthetically versatile bicyclic system allows for the introduction of multiple substituent variations in our search for good ER ligands. In contrast to our initial design expectations, however, we found that introducing a 6-hydroxyl into the core resulted in reduced binding affinity, which suggested that the ligand core is binding to the ER in a reversed fashion, with a pendant phenol, rather than the hydroxyl pyrimidine group, serving as the mimic of the A-ring of estradiol. Further work based on the new lead compound, **12c**, has led to ER ligands with higher binding affinity.[17]

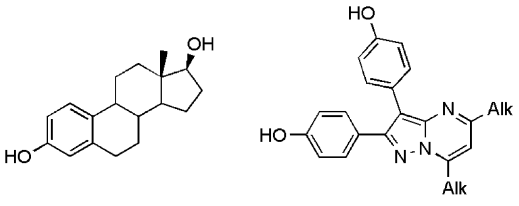


Figure 3. Redesigned pyrazolo[1,5-*a*]pyrimidine systems based on lead compound **12c**.

Acknowledgements

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