Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 947–950

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Diaryl-Dialkyl-Substituted Pyrazoles: Regioselective Synthesis and Binding Affinity for the Estrogen Receptor

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Received 19 November 2001; accepted 18 January 2002

Abstract—We have developed two novel series of tetrasubstituted pyrazoles, embodying 1,3-diaryl-4,5-dialkyl or 3,5-diaryl-1,4-dialkyl substitution patterns. The scope of a regioselective method, developed by us earlier, was expanded to allow the synthesis of the first series of these tetrasubstituted pyrazoles directly from α,β-unsaturated ketones. The binding affinity of some of these pyrazoles for the estrogen receptor (ER) subtypes ERα and ERβ is very high, and the overall affinity pattern suggests the importance of three phenol substituents for high affinity, ERα-selective binding.

The estrogen receptor (ER) has the capacity to bind a wide variety of non-steroidal ligands with high affinity.[1] This feature has stimulated the development of ligands that act as selective estrogen receptor modulators (SERMs, i.e., compounds that have a mixed endocrine profile that affords agonistic or antagonistic activity in a tissue-specific manner.)[2] Some of this selectivity may derive from differential binding to the two ER subtypes, ERα and ERβ.[3] Compounds in this class are of use for menopausal hormone replacement and the prevention and treatment of breast cancer.[4]

The tetrasubstituted pyrazole core has emerged as a target of particular interest in our efforts to develop SERMs with high affinity and selectivity for the ER subtypes.[5] We have found that some 1,3,5-triaryl-4-alkyl substituted pyrazoles, such as the propylpyrazole triol **PPT** (Fig. 1), have very high ERα-selective binding affinity and potency;[5] others have found that differently substituted pyrazoles have ERβ selectivity.[6] Molecular modeling and phenol deletion/binding affinity studies suggest that the C(3) phenol of the 1,3,5-triaryl-4-alkyl substituted pyrazoles we have studied acts as the mimic of the estradiol A-ring (Fig. 1).[5] A comparable orientation appears to be adopted by the core isomeric 1,3,4-triaryl-5-alkyl pyrazoles (Fig. 1).[7]

In our continuing interest in understanding the structural determinants of the ERα binding selectivity of these pyrazoles, we envisioned the synthesis of compounds having a 1,3-diaryl-4,5-dialkyl or 3,5-diaryl-1,4-dialkyl substitution patterns (Fig. 1, Series I and II, respectively). The motivating rationale behind the design of these new pyrazole ligand series consisted of (a) keeping the C(3) phenol as a putative A-ring mimic of estradiol, (b) while modifying other portions of the pyrazole encompassing substituents at N(1), C(4), and C(5), thereby probing the compliance of the region of the ligand-binding pocket with which these substituents interact and the degree to which these changes affect ERα selectivity. Herein, we report the synthesis of tetrasubstituted pyrazoles having two new substitution patterns and the evaluation of their ERα and ERβ binding affinity (Fig. 1). In the process, we have expanded the scope of a regioselective synthesis route that allows the first of these series pyrazoles to be prepared directly from simple α,β-unsaturated ketone precursors.

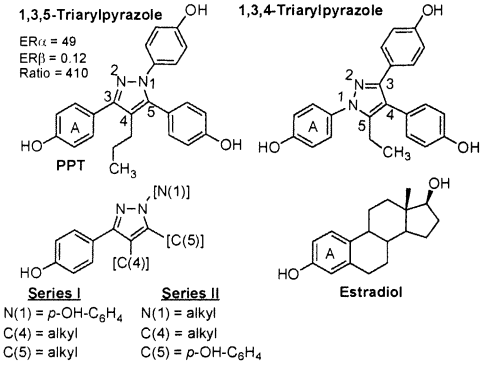

Figure 1.

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0960-894X/02/$ - see front matter
PII: S0960-894X(02)00057-4

A convenient synthesis of the pyrazole core involves the condensation of 1,3-diketones with hydrazines. This method is most effective when the diketone is symmetrical, [i.e., when the C(3) and C(5) substituents of the pyrazole are equivalent][8] However, when the 1,3-dione is unsymmetrical, two regioisomers are produced, and these can be very difficult to separate.[9] We have developed a regioselective approach to tetrasubstituted pyrazoles that produces a single regioisomer starting from a chalcone (cf. Scheme 1).[9] Recently, Katritzky[10] has reported a related method that utilizes a 1-benzotriazolyl activating substituent at the α-position of the chalcone.

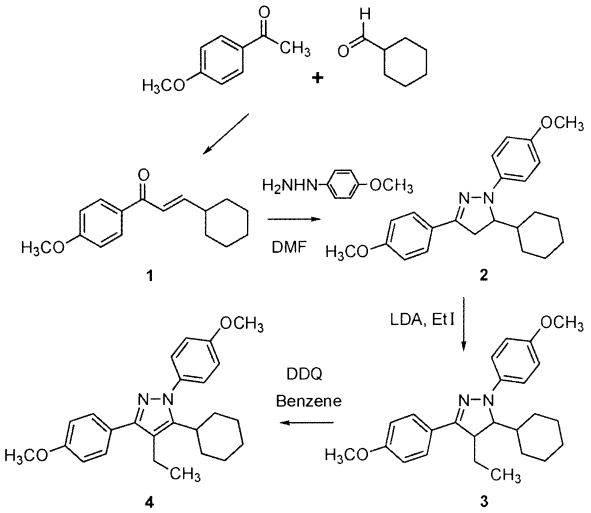
In the initial investigations of our method,[9] we were unable to produce the tetrasubstituted pyrazoles directly, because chalcones *substituted at the α position* with alkyl groups proved to be too unreactive in the initial hydrazine condensation step. However, hydrazine condensation with a chalcone *unsubstituted at the α position* proceeded effectively, and the resulting pyrazoline intermediate could be alkylated at C(4) and oxidized, giving the tetrasubstituted pyrazole.[9] Our synthesis of the Series I targets (i.e., 1,3-diaryl-4,5-dialkyl pyrazoles) was, therefore, envisioned to proceed by this route, as shown in Scheme 1.

Attempts to synthesize the alkylidine acetophenone from commercially available *p*-methoxyacetophenone and cyclohexanecarboxaldehyde under various basic conditions (NaOH, KOH, LDA) gave enone **1**, but in low yield (e.g., NaOH, 18% yield). Reaction of enone **1** with *p*-methoxyphenylhydrazine hydrochloride gave two major products, believed to be pyrazole and pyrazoline intermediates. However, attempts to isolate the pyrazoline and alkylate it using LDA and ethyl or propyl iodine, gave the non-alkylated, oxidized pyrazoline as the major product. The difficulties in handling this pyrazoline compounded by low yields in the enone formation prompted us to search for a more efficient sequence.

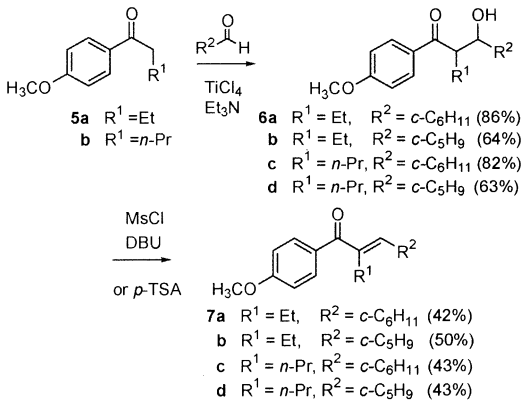
One option to circumvent the problems we encountered in this approach would be to introduce the C(4)-alkyl group at an earlier stage of the synthesis, by starting from α-alkyl-substituted enones. (As noted above, we had been unable to effect the hydrazine condensation with α-alkyl-substituted chalcones.[9]) There is literature precedent for the synthesis of α *alkyl-substituted* chalcones from aryl ketones and aryl aldehydes under gaseous HCl conditions.[11] However, our attempts to adapt this synthesis to produce the α-substituted-α-alkylidene acylphenones (e.g., **7a–d**) directly by the reaction of acylphenones **5a**,**b** and cyclohexane carboxaldehyde with anhydrous HCl were unsuccessful, and condensation under basic conditions (NaOH, KOH, NaOMe) gave very poor yields.

We found that the desired α-alkylidene butyro- and valerophenones (**7a–d**) were synthesized satisfactorily in two steps, aldol condensation and then dehydration (Scheme 2). The use of Lewis acids for the asymmetric synthesis of secondary alcohols from propiophenone and an aldehyde, in the presence of a base, is widely known.[12] Activation of ketones **5a**,**b** with $TiCl_4$, followed by enolate formation and condensation with the cycloalkane aldehydes, gave the aldol products **6a–d**. The stereochemistry of the aldol products was not determined, because they were simply dehydrated (MsCl/DBU or *p*-TSA) to give the desired enone products **7a–d**, together with some retro-aldol byproducts.

Despite our earlier lack of success in reacting α-substituted chalcones with substituted hydrazines,[9] we found that the α-alkylidine acylphenones (**7a–d**) did undergo reaction with *p*-methoxyphenylhydrazine hydrochloride in DMF in a slow reaction affording a crude mixture of pyrazoline (**8a–d**) and pyrazoles (**9a–d** and **10a–d**) products (Scheme 3). This mixture was stirred in benzene and DDQ to afford the pyrazoles, from which the desired isomers (**9a–d**) were isolated in 30–50% overall yields. The surprising formation of small amounts (ca. 10%) of undesired isomeric pyrazole side products (**10a–d**) appears to be the result of hydrazine condensation on an enone precursor that had undergone double bond isomerization from the α,β- to the alternate α,β′-position under the acidic reaction conditions. Although these products (**10a–d**) are constitutional isomers of the desired pyrazoles **9a–d**, they are easily removed from them by chromatography; this was not the case with many of the regiosiomers that are



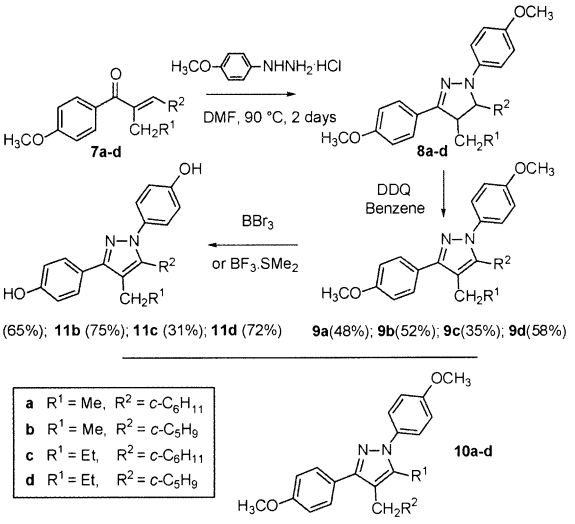
Scheme 1.



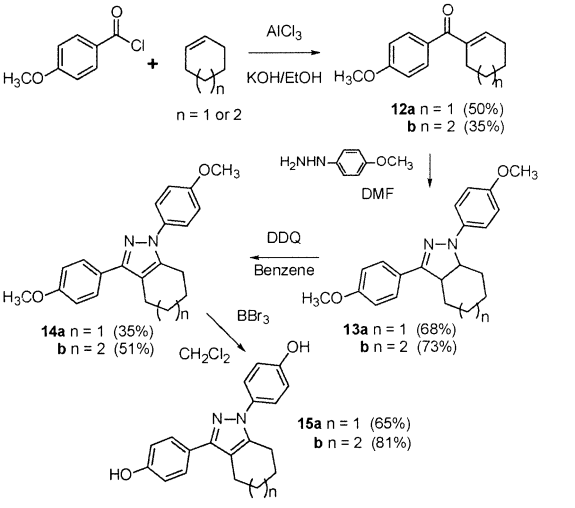
Scheme 2.

obtained by hydrazine condensation with unsymmetrical 1,3-diketones. The pyrazoles (**9a**–**d**) were then deprotected to give the final phenol products (**11a**–**d**).

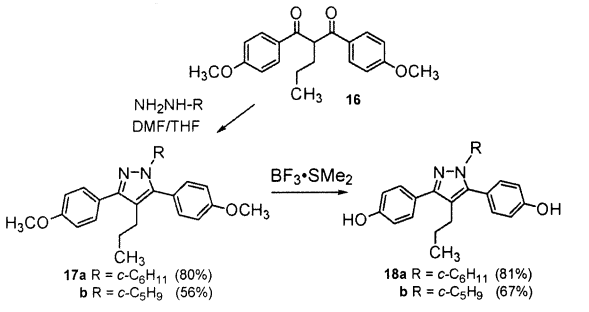
Another set of Series I pyrazoles consisted of those having a more rigid core, with a six- or seven-membered ring fused across the C(4) and C(5) positions. The synthesis of such compounds (Scheme 4) started with a Friedel–Crafts acylation of cyclohexene or cycloheptene



Scheme 3.



Scheme 4.



Scheme 5.

with anisoyl chloride to give the corresponding 1-aroylcycloalkenes (**12a**,**b**), followed by condensation with *p*-methoxyphenylhydrazine hydrochloride salt to give the pyrazolines (**13a**,**b**). Oxidation with DDQ in benzene and deprotection with BBr_3 afforded the desired pyrazoles (**15a**,**b**).

The targets for the Series II type compounds began with the introduction of a non-aromatic substituent at N(1) by reacting the symmetrical 1,3-diketone **16**[8] with cyclopentyl or cyclohexyl hydrazine to form the desired pyrazole core directly. Deprotection with $BF_3{\cdot}SMe_2$ gave the desired products (**18a**,**b**) (Scheme 5).

The binding affinity for each pyrazole phenol for human estrogen receptors alpha and beta (ERα and ERβ) was determined using a competitive radiometric assay; affinities were expressed relative to that of estradiol, to give relative binding affinity (RBA) values (Table 1).[13]

All pyrazoles that we have studied in the past have shown ERα-binding selectivity.[5] This is also a feature of most of the new pyrazoles studied here. It is curious, however, that the pyrazoles having the highest affinity, the N(1)-cycloalkyl compounds of Series II (**18a**,**b**), were also those that showed only very low ERα/ERβ selectivity. These two pyrazoles, in fact, are among the highest affinity ER ligands in this heterocyclic class, a result that is consistent with prior indications that a variety of substituents, even a *t*-butyl group, is tolerated at the N(1) position of these pyrazoles.[5,7,8] By contrast, the very low ERα selectivity of the pyrazoles with these N(1) cycloalkyl groups was unexpected and indicates that they must be interacting with the receptor in a manner that does not discriminate in binding affinity between ERα and ERβ (see below).

Lowest affinities were shown for the Series I pyrazoles with the cycloalkyl system fused at C-4,5 (**15a**,**b**). The other Series I pyrazoles having alkyl groups at C(4) and C(5) (**11a**–**d**) had intermediate affinity, but typically less than that shown previously by the triaryl pyrazoles of the 1,3,5- and 1,3,4-substitution patterns (cf. Fig. 1).[5,7] Both sets of Series I pyrazoles show substantial preferential binding to ERα. Together, these data suggest that the phenolic substituent on C(5) of the pyrazole does play an important role in binding affinity, potentially as the hydrogen bonding partner of His524 (see Fig. 2). When this phenolic substituent is replaced with

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

Compd	ERα	ERβ	α/β
11a	0.59	0.14	4.2
11b	2.1	0.23	8.9
11c	1.1	0.11	9.4
11d	3.6	0.60	6.0
15a	0.56	0.042	13
15b	0.19	0.047	4
18a	30	13	2.3
18b	74	71	1.0

[a]Relative binding affinity (RBA) values, where estradiol = 100.

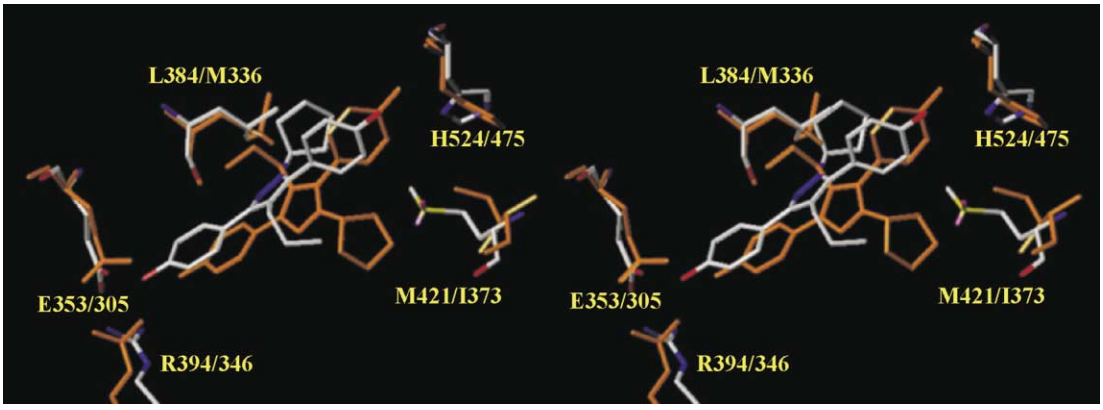


Figure 2. Crossed-stereo view of a model of pyrazole **18b** in the ligand binding pocket of ERα and ERβ. Atom colors are used for the ligand and residues of ERα, and orange for those of ERβ. Residue names and identities are given as ERα/ERβ.

an alkyl group (as in compounds **11a**–**d** and **15a**,**b**), affinity drops to a large degree.

We have previously used molecular modeling to examine the orientation of pyrazole binding in the ER ligand binding pocket and to try to rationalize ERα/ERβ selectivity.[5,14] Using the same methods, we addressed three issues with the following outcomes: (1) The very low affinity of the C(4,5) fused cycloalkyl pyrazoles **15a**,**b** appears to result from a very poor fit in the binding pocket (not shown); (2) the lower affinity of the C(4),C(5)-dialkyl pyrazoles **11a**–**d** compared to **PPT** appears to result from the loss of the interaction with the helix-11 histidine (524 in ERα and 475 in ERβ) when the C(5) phenol is replaced by an alkyl group (not shown), and (3) the high affinity but low ERα/ERβ appears to result from alternative binding orientations in the two ER subtypes (Fig. 2). When pyrazole **18b** is minimized in the ligand pocket of ERα and ERβ, different ligand orientations are found that are related by a 'rotation' about an axis through the two phenols. In both cases, the ligand fits well in the pocket and enjoys productive interactions with the A-ring (E353/305; R394/346) and D-ring (H524/472) hydrogen bonding partners. By flipping, this ligand appears to avoid unproductive interactions with the residues in the ligand pocket that differ between ERα and ERβ (L384/M336 and M421/I373).

In conclusion, we have extended a regioselective method for the synthesis of tetrasubstituted pyrazoles and used it to prepare pyrazole ligands for the estrogen receptor that have new patterns of diaryl and dialkyl substitution, some of which have very high affinity for both ERα and ERβ, in contradistinction with the high affinity ERα-selective pyrazoles in the triaryl series. The lack of subtype selectivity of some of these pyrazoles might be the consequence of alternative orientations that they adopt in the binding pocket of the two ER subtypes.

Acknowledgements

This work was supported by a grant from the National Institutes of Health (PHS 5R37 DK15556). We are grateful to Kathryn Carlson for biological assays and Dorina Kosztin for assistance with molecular modeling studies.

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