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Synthesis and evaluation of 4-(substituted styryl/alkenyl)-3,5-bis(4-hydroxyphenyl)-isoxazoles as ligands for the estrogen receptor

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Introduction

ABSTRACT

A series of 3,5-bis (4-hydroxyphenyl) isoxazoles bearing a styryl/alkyl vinyl group at the 4-position were prepared and evaluated as ligands for the estrogen receptor-alpha (ER α). The target compounds were prepared using the Suzuki reaction to couple an iodo-isoxazole intermediate with a series of styryl/alke-nyl boronic acids, followed by O-demethylation. The products were evaluated for their estrogen receptor- α ligand binding domain (ER α -LBD) binding affinity using a competitive binding assay. The 4-(4-hydroxy-styryl) derivative 4 h displays binding properties similar to those of the previously described pyrazole class of ER ligands, indicating that the ER α -LBD tolerates the presence of the added vinyl group at the 4-position of the isoxazole ring.

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The estrogen receptor displays the capacity to bind a wide variety of nonsteroidal ligands and to generate diverse biological responses as a consequence of that binding interaction.¹ A number of investigators have undertaken efforts to systematically prepare and evaluate nonsteroidal ligand cores. The strategy, exemplified by the work of Katzenellenbogen and others, typically involved the preparation of a bis(4-hydroxyphenyl) heteroarene to mimic the estradiol skeleton and the incorporation of additional functional groups (R_1/R_2) that would enhance ER affinity and influence efficacy^{2–18} (Fig. 1). Relative binding affinities (RBA) for these derivatives ranged from 0.01% to 140% (compared to estradiol—100%), depending both upon the nature of the heteroaryl group and the R_1/R_2 substituents.

As part of our program to identify new structural motifs that elicit the desired ER response, we also focused on the preparation of new di- and tri-aryl heteroarenes. Although 3,5-diaryl isoxazoles are electronically similar to the 3,5-diaryl pyrazoles, isoxazole analogs had not been as extensively evaluated for estrogen ligand development as the corresponding pyrazoles.^{10,18} We noted that incorporation of a CH_2 -, C=O or CH_2O -linker between the



Figure 1. Nonsteroidal ER ligands based on bis(4-hydroxyphenyl) heteroarene scaffold and incorporating additional R1 and R2 substituents.

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Figure 2. Transformation of 4-substituted-3,5-bis(4-hydroxyphenyl) isoxazoles to corresponding 4-styryl/alkylvinyl-3,5-bis(4-hydroxyphenyl) isoxazoles.

isoxazole ring and the aryl group gave products with significant ER affinity, however, none of the derivatives had employed a vinyl group to link the isoxazole core with the terminal (substituted phenyl) moiety. We hypothesized that an appropriate styryl/alkyl-vinyl group would yield derivatives that retain significant affinity for the ER α -LBD (Fig. 2). In addition, the synthetic approach should contain elements that would make it amenable to combinatorial chemistry, thereby permitting rapid exploitation of the chemical space. This paper describes the synthesis and receptor binding results with this series of new 4-(styryl/alkenyl)-isoxazole derivatives.

Results and discussion

Our synthetic objective involved the development of a direct, reliable synthetic route to the preparation of the target compounds. We examined two approaches, based upon a 4-iodo-3,5-bis(4-methoxyphenyl)-isoxazole intermediate, either of which could be expanded to combinatorial/parallel synthesis. In the first approach (Scheme 1) we converted the 4-iodo intermediate 1^{18} to the 4-vinyl boronic acid derivative **2** using the Sonogashira reaction, ^{19,20} followed by catechol hydroboration and hydrolysis. Subsequent Suzuki coupling with substituted aryl halides gave 4-(substituted styryl)-3,5-bis(4-methoxyphenyl) isoxazoles **3**. The Sonogashira reaction, however, proceeded in low yield (~30%) and was accompanied by side products that required careful separation. Although the yield for the hydroboration step was

satisfactory (77%), the yields for the subsequent Suzuki reactions to give the 4-(substituted styryl)-3,5-bis(4-methoxyphenyl) isoxazoles **3** were low (20–40%). Therefore we developed the second approach, (Scheme 2) in which we coupled the 4-iodo intermediate **1** with a series of commercially available substituted styryl and alkylvinyl boronic acids. In this case, the yields for the Suzuki reaction were very good (56–98%). Subsequent O-demethylation of the intermediates **3a–m**, using boron tribromide or aluminum trichloride-ethyl mercaptan gave the initial series of target compounds **4a–m** in good yields (53–98%).

Although the second approach was synthetically more successful, it is limited by the availability of coupling partners, that is, arylvinyl boronic acids are not as readily available as aryl halides. As a basis for chemical library design, we demonstrated that other arylvinyl boronic acids could be readily accessed for use in this synthetic strategy. Conversion of aryl halides to the corresponding aryl acetylenes using the Sonogashira reaction has been well described. For our study, we used three such alkynes and converted them to the corresponding aryl vinyl boronic acids²¹ and subsequently to the isoxazole derivatives (Scheme 3). The yields for the catechol hydroboration, hydrolysis (43–61%) and coupling steps for the representative compounds **5n–p**^{22–24} were very satisfactory (43–61%). Conversion to the isoxazole derivatives **3n–p** also proceeded in good yields (73–93%).

The initial series of 4-(substituted styryl/alkenyl)-3,5-bis(4-hydroxyphenyl)isoxazoles **4a–4m** were evaluated as ligands for ER α -LBD using a competitive binding assay.^{25,26} The relative



Scheme 1. Synthesis of 4-styryl-3,5-bis(4-methoxyphenyl) isoxazoles 3 via the 4-vinylboronic acid intermediate. Reagents and conditions: (i) Trimethylsilyl acetylene, TEA, Cu(1)I, [P(C₆H₅)₃]₄Pd(0); (ii) TBAF, THF; (iii) catechol borane, reflux; (iv) aryl iodide, Pd(P(C₆H₅)₃]₂Cl₂, DME, 90 °C,16 h.



Scheme 2. Synthesis of 4-styryl-3,5-bis(4-hydroxyphenyl) isoxazoles **4a–4m** via the 4-iodo intermediate and commercially available styryl/alkylvinyl boronic acids. Reagents and conditions: (i) Ar/R–CH=CH–B(OH)₂–1.5 equiv, Pd(P(C₆H₅)₃)₂Cl₂, NaHCO₃, H₂O–DME (ii) BBR₃, CH₂Cl₂ (iii) AlCl₃ (5.8 equiv), C₂H₅SH (4.9 equiv), CH₂Cl₂.



Scheme 3. Synthesis of novel arylvinyl boronic acids 5n-p and conversion to 4-(substituted styryl)-3,5-bis(4-methoxyphenyl) isoxazole derivatives 3n-p. Reagents and conditions: (i) Catechol borane; (ii) H₂O; (iii) 1, Pd(P(C₆H₅)₃)₂Cl₂, NaHCO₃, H₂O-DME.

Table 1

Relative binding affinities (RBA) of isoxazole derivatives for the estrogen receptor- α ligand binding domain (ER α -LBD)



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Compound	R	RBA ± S.D
4a	E-C ₆ H ₅	3.5 ± 1.0%
4b	$Z-C_6H_5$	8.7 ± 1.2
4c	$4-CH_3-C_6H_5$	$0.6 \pm 0.2\%$
4d	4-F-C ₆ H ₅	3.1 ± 0.7%
4e	4-Cl-C ₆ H ₅	$0.8 \pm 0.2\%$
4f	$4-CF_3-C_6H_5$	5.3 ± 1.2
4g	$3-CF_3-C_6H_5$	1.5 ± 0.7
4h	$4-HO-C_6H_5$	16.4 ± 3.8
4i	3-HO-C ₆ H ₅	8.7 ± 1.5
4j	<i>n</i> -C ₇ H ₁₅	1.8 ± 1.2
4k	<i>n</i> -C ₈ H ₁₇	2.5 ± 0.7
41	$n - C_9 H_{19}$	1.3 ± 0.6
4m	$t-C_4H_9$	4.3 ± 3.2

RBA = 100 × [E]/[C] where [E] is the concentration of unlabeled estradiol necessary to reduce the specific binding of [³H]-estradiol to the ER α -LBD by 50% and [C] is the concentration of the competitive ligand necessary to reduce specific binding by 50%. The RBA of estradiol is 100% at 25 °C. Curves for ligand and estradiol had correlation coefficients >95%.

binding affinity (RBA) values for the new compounds are shown in Table 1, where estradiol has a value of 100%. All of the compounds demonstrated significant affinity for the ER α -LBD, with values ranging from <1% to 16% that of estradiol. The highest affinity was observed for the 4-hydroxystyryl derivative **4h** (16.4%), with the 3-hydroxy isomer **4i** (8.7%) slightly lower. Substitution of the ring at the 4-position with alkyl or halogenated groups (**4c–g**) reduced binding affinity (RBA <1–5.3%) and as did replacement of the aromatic ring by alkyl groups (**4j–m**, RBA = 1.3–4.3%). The *Z*-styryl isomer **4b** appeared to be a slightly better ligand for ER α -LBD than the *E*-isomer **4a** (8.7% vs 4%). Because substitution on the phenyl ring with groups other than hydroxyl appeared to reduce the binding affinity, we did not convert isoxazole derivatives **3n–3p** to the corresponding bis(4-hydroxyphenyl) analogs **4n–4p** for evaluation.

The binding properties of this series of tri-substituted isoxazoles compares favorably with previous studies on tris(4-hydroxyphenyl) five-membered heterocycles. For example, the Chiron patent¹⁸ reported a relative binding affinity (RBA) of 30% for 3,4,5-tris(4-hydroxyphenyl) isoxazole. In our case, introduction of the vinyl group between the isoxazole ring and the aryl group at the 4-position appears to be well tolerated in terms of ER α -LBD binding. The binding of the new compounds also compares favorably with the other heterocyclic analogs. Most of the tris(4-hydroxyphenyl) substituted pyrazoles reported by Katzenellenbogen also have RBA values in the 5–25% range.^{3,5,8,10,11} Figure 3 presents the RBA value for **4h** compared to two of the more potent isoxazole and pyrazole compounds.

The RBA values for the 4- and 3-hydroxyphenyl derivatives **4h** and **4i** are also comparable to the highest values observed for the tri- and tetrasubstituted pyrrole ER ligands recently reported by Gust.^{14,15} It should be noted that pyrazoles and furans which have higher reported RBA values than **4h** may achieve the enhanced affinity through the additional fourth substituent. This is not possible with the isoxazole scaffold. By comparison, the substituted isoxazoles described in this study generally were better than the previously reported imidazoles, oxazoles and thiazole which had very low RBA (<1%) values for ER.¹⁰

Based prior studies of the interaction between nonsteroidal ligands and ER α -LBD, we proposed a binding mode for the new ligands that provides a rationale for the structure-activity relationship. We illustrated this mode using compound 4d whose RBA value is less than that of the highest affinity agent **4h** but somewhat more than the less active alkyl vinyl derivatives.²⁷ As with most nonsteroidal ligands of this type, the 3,5-bis(4-hydroxyphenyl)-isoxazole scaffold mimics the internal steroidal core of estradiol. The vinyl moiety at the 4-position experiences torsional strain due to the neighboring (4-fluorophenyl) groups and adopts a non-coplanar conformation that will reduce the steric strain. The more distal vinyl-aryl bond can also undergo rotation to achieve the most effective ligand-ERa-LBD contacts and further lower the energy of the complex. Our study (Fig. 4) suggests that the terminal 4-fluoro-group interacts with residues Leu-540(helix-12) and Trp-383(helix5), as well as Thr-347 (helix-3), which is not see in this depiction. Hydrogen bonding by either the 4-hydroxyl (4h) or 3-hydroxyl (4i) group provides additional stabilization within this pocket. Hydrophobic substituents such as choro- (4e), methyl (4c) or trifluoromethyl (4f,4g) are tolerated but not favored. The alkyl vinl derivatives **4i-4m** are also accommodated within this hydrophobic pocket, but provide no additional interactions that would strengthen binding. Other modeling studies have identified Thr-347 as the residue in the 11ß-pocket most likely to establish hydrogen bonding with the distal phenolic hydroxyl.^{4,5} This would appear to be the case as the two ligands with the highest RBA values were the 4- and 3-hydroxystyryl isoxazoles 4h and 4i. Substitution with non-hydrogen bonding or hydrophobic



Figure 3. Comparison of compound 4h with similar tris(4-hydroxyphenyl) heteroarenes.



Figure 4. Docking study with isoxazole 4d and ERα-LBD showing interaction between 4-(4-fluorophenyl)-group and Leu-540 and Trp-383 residues.

groups reduces the binding interaction, but conformation mobility may result in alternative binding modes that generate stable complexes. One interesting observation in this series was the difference between the 4-*E*- and 4-*Z*-styryl isoxazoles where the *Z*-isomer **4b** had a slightly higher affinity than the *E*-isomer **4a** (8.7% vs 4%). It is possible that the terminal phenyl group of the Z-derivative can access a slightly different and/or more favorable binding pocket than the *E*-counterpart.

Conclusions

In this study we developed an approach to the synthesis of 4-(substituted styryl)-3,5-bis(4-hydroxyphenyl) isoxazoles that is efficient and can be extended to combinatorial expansion. Although limited by the availability of the substituted styryl boronic acids, such reagents can be prepared from commercially available starting materials. The resultant products demonstrate significant binding affinity for the ER α -LBD that is comparable to previously described bis- and tris-(4-hydroxyphenyl) isoxazole, pyrazole and furan derivatives. The presence of the vinyl group at the 4-position is well tolerated and may permit the distal 3-/ 4-hydroxyphenyl moiety, present in compounds **4h** and **4i**, to adopt conformations that can access complementary residues, such as Thr-347, Trp-383, and Leu-540 in the 11ß-binding pocket. The results provide the basis for future studies that evaluate the relationship between the 4-substituent and estrogenic potency. Studies in which the 4-styryl group is substituted with dialkylaminoethoxy groups are in progress as well.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012. 06.097.

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