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TITLE: Solid Phase Combinatorial Approach to Estradiol Tamoxifen/Raloxifene Hybrids: Novel Chemotherapeutic/Prophylactic Selective Estrogen Receptor Modulators

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<td>The objective of this project is the development of new chemotherapeutic agents for the treatment of hormone-responsive breast cancer using a solid phase approach to synthesize new agents having features common to both steroids and antiestrogens. Previously we functionalized the carboxy resin with both the E- and Z-tributylstannylvinyl estradiol, and prepared an initial series of iodophenoxyalkylamines that will be coupled to the resin-bound steroid. Coupling reactions with the Z-stannylvinyl estradiol were generally unsuccessful on solid-phase and coupling with the E-isomers proceeded in low yields. We have prepared more iodophenoxyalkylamines and are preparing the target compounds via solution phase methods. We are exploring an approach using resin-bound estradiol vinylboronic acids as an alternative method.</td>
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Copies of posters of GRC/ACS- three(3)
4. Introduction.

The overall objective of this project is the development of new chemotherapeutic agents for the treatment or prevention of hormone-responsive breast cancer. Our approach involves the solid-phase synthesis of a series of 17α-(substituted-phenyl)vinyl estradiols in which the substituent is derived from the anti-estrogen imparting components of tamoxifen and raloxifene. The new compounds would be evaluated by appropriate biological assays to determine the receptor binding affinity and efficacy. The results would be evaluated to determine the targets for subsequent synthetic efforts designed to enhance the biological properties of the substances. This report describes the efforts made during the past year to achieve those objectives.
5. Body

The research proposal described 5 specific tasks in the Statement of Work. These were: 1. Initial target compound design. 2. Chemical synthesis of target compounds in initial directed library. 3. Measurement of biological properties (receptor affinity and efficacy). 4. Assessment of structure-activity relationships. 5. Chemical synthesis of target compounds in second generation libraries. The completion of the first task was described in the report last year. Work on the second and third tasks continued during this past year and will be described in this report.

Task 2. Chemical synthesis of target compounds in initial directed library. (Months 1-24).

During this period we focused on two aspects. The first was continued preparation of the series of dialkylaminoalkoxylphenyl iodides that constitute the coupling partners for the solid phase Stille reaction. The second was the synthesis of the target compounds on solid phase followed by cleavage, purification and characterization.

The synthesis of virtually all of the dialkylaminoalkoxyphenyl iodides in the ethoxy- and propoxy series has been completed. The ethoxy- series was achieved in good yields (75-85%) in one step from the commercially available hydroxyethyl amines and the iodophenols using the Mitsunobu reaction. The propoxy-series was prepared in two steps from bromopropanol and the iodophenol (Mitsunobu reaction) followed by reaction with the appropriate dialkyl amine. Overall yields were lower (50%) but still satisfactory. Preparation of the butoxy-series is in progress using the second method. The products, as their oxalate salts, are available for the subsequent coupling reaction.
The Stille coupling of the iodophenyl ethers and the resin-bound E- and Z-tri-butylstannylvinyl estradiols was undertaken using the procedure employed for the synthesis of the simpler substituted phenylvinyl estradiols. Reactions with the E-isomer gave low yields of product along with a mixture of by-products. The reactions were repeated without being able to significantly improve the yields. Sufficient quantities of the dimethylaminoethoxyphenyl-vinyl estradiol were obtained to submit for biological evaluation. Reactions with the Z-isomer gave no characterized product. This observation was similar to what we had obtained with some of the solution couplings with the Z-isomer.

In order to obtain sufficient material in the target series we have temporarily reverted to the solution based chemistry. We are concentrating on the E-isomers because they can be obtained more reliably, in higher yield and they are chemically more stable. We are also exploring the use of the Suzuki coupling reaction and so have done preliminary work in preparation and coupling of vinyl boronic acids. In order to preserve the more valuable ethynyl estradiol starting material, we have used a simpler estrogenic core [3,5-bis-(4-hydroxyphenyl)-isoxazole] described by Katzenellenbogen, as a model system. We have been able to prepare phenyl vinyl derivatives via two approaches using this scaffold and are now applying this methodology to the ethynyl estradiol series. We have started to prepare the estradiol vinylboronic acids and esters in preparation for both the Suzuki solution and solid phase organic syntheses. While the initial work will be done using solution chemistry, we will keep in mind the application to solid phase organic synthesis.


We have continued to develop the biological evaluative methods for the new compounds. As described in the first report we have established the assays for determining the receptor binding affinity utilizing the ligand binding domain overexpressed in a bacterial cell line. The initial evaluation was with the ER-alpha-LBD, although we have been able to extend this to the ER-beta-LBD as well. We
used these two ER-LBDs to evaluate the model isoxazoles prepared as part of our boronic acid study. We also have evaluated the first of the dialkylaminoalkoxyphenylvinyl estradiols to begin the comparison of the target compounds versus the simpler phenylvinyl estradiols.

We have also started the evaluation of the isomeric E-/Z-substituted phenylvinyl estradiols (6 compounds per series) in the immature female rat uterotrophic growth assay. Such assays involve 280 rats per study in order to be able to do a direct comparison of the compounds. We had found that we could not obtain the same results by pooling data from separate assays. In these recent assays, we have observed that the uterotrophic data do not always correspond to the binding data. So far, for the 5 series that we have evaluated, the ortho-substituted phenyl vinyl compounds (both E- and Z-isomers) usually are the most active. Also, the simple substituted phenylvinyl compounds are all agonists (estrogenic). Therefore, as we proceed to the dialkylaminoalkoxyphenyl vinyl series, we hope to observe a transformation to antagonist (anti-estrogenic) properties.

To enhance our ability to assess both affinity and efficacy we are starting to generate the stably transfected ERα/β-LBDluciferase assay. This will allow us to determine simultaneously the affinity and efficacy of the new compounds much more rapidly than currently possible.

**Task 4. Assessment of structure-activity relationships (Months 6-24).**

We have started to develop the structure-activity relationships for the 17α-(substituted-phenyl)vinyl estradiols. In conjunction with the other projects we have undertaken the molecular modeling docking studies with the ligands and the ER-LBD. Our initial molecular dynamics docking studies with the para-substituted phenyl vinyl estradiols gave a linear relationship between the calculated binding energies and the relative binding affinities (RBA). The studies also suggest that the
region into which we are introducing the dialkylaminoalkoxy-side chains should be able to accommodate the substituent.

The evaluation of the in vivo data suggests that the simpler derivatives are full agonists with potencies ranging from more active than estradiol to less than 1% as potent as estradiol. In most, but not all cases, the ortho-isomer in both the E- and Z-series is the most active. In the E- series, the meta- and para-isomers are generally, but not always, weak estrogenic agonists. In the Z-isomers, the meta- and para-isomers are quite active, but not as potent as the ortho-products.


- Completed preparation of most dialkylaminoalkoxyphenyl iodide coupling reagents
- Developed molecular dynamics methods for evaluating ligand binding energies and RBA
- Developed in vivo uterotrophic assay and in vitro transfection luciferase assay
- Synthesized phenylvinyl derivatives of diaryl isoxazoles as models for alternate boronic acid approach
- Completed initial SAR studies for simple para-substituted phenylvinyl estradiols
7. Reportable Outcomes.

a. Manuscripts, abstracts, presentations


5. Several manuscripts are in progress in which the material presented in the posters will be described in greater detail.

b. Degrees obtained supported by the award.

8. Conclusions.

At this point, we are continuing to make progress on completing our ultimate objectives. We have had difficulty translating our initial success in synthesizing simpler estrogens on solid phase to the preparation of more complex compounds. We have continued to prepare the key reagents and develop alternatives, including solution based syntheses. We have expanded our biological assays to include in vivo uterotrophic growth assays and an in vitro transfection assay. Preliminary biological results indicate that simpler estrogenic derivatives retain full receptor potency. Molecular dynamics studies demonstrate a direct relationship between calculated binding energies and observed binding affinities. For the next year we will continue to prepare the initial series of target compounds and evaluate their estrogen receptor-related properties.

9. References.

None.

10. Appendix.

The appendix material consists of copies of the 3 posters for the presentations at the Gordon Conference and at the ACS meeting.
Preparation and Evaluation of Isomeric series of 17α-(Substituted-phenyl)vinyl Estradiols

Robert N. Horsford*, Choon Young Lee†, Carolyn J. Friel*, Robert Dilis*, Alun Hughes*, and Eugene R. Desombre*

Department of Chemistry* and Pharmaceutical Sciences†, Northeastern University, 360 Huntington Avenue, Boston, MA 02115 and The Ben May Institute for Cancer Research*, The University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637

Introduction:

The estrogen receptor (ER) is a member of the superfamily of nuclear receptor orphan (NUR) receptors, which is characterized by an essential steroid hormone binding domain of the classical steroid/thyroid hormone factor-1 domain, a DNA binding domain (DBD), and a ligand binding domain (LBD), and is a central estrogen receptor (ER) domain. ERs have been shown to be one of the most active receptors in the cell, with an important role in the development of the entire cell cycle. However, the estrogen receptor is implicated with many normal physiological functions, including sexual development, reproduction, and bone metabolism, as well as lipid metabolism, and is regulated by estrogen. It has been shown that the main mechanism of action of ER-active compounds is through binding to the estrogen receptor, in a process known as the hormonal response mechanism. In addition, the expression of certain ER ligands is regulated by the estrogen receptor.

In recent years, a large amount of research has been focused on the development of estrogen receptor ligands that would not interfere with the normal physiological functions of the estrogen receptor. As part of our program to develop potential ligands for the estrogen receptor, we have prepared three and similar compounds as potential modulators and have found that some of the compounds exhibit interesting binding properties. The results of our study are presented in this paper. Further work is ongoing to explore the potential of these compounds as modulators of the estrogen receptor.

![Figure 1](image1.png)

Figure 1: Synthesis of 17α-(Substituted-phenyl)vinyl Estradiols

The compounds were synthesized using the general scheme shown in Figure 1. The 17α-(Substituted-phenyl)vinyl Estradiols were prepared by the method of Desombre et al. (1985) and modified by adding the substituent to the double bond. All compounds were characterized by 1H and 13C NMR, and their elemental analyses were consistent with the proposed structures.

![Figure 2](image2.png)

Figure 2: Structures of 17α-(Substituted-phenyl)vinyl Estradiols

Table 1: Comparison of Binding Affinities (RBA) for Compounds in Study

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<tr>
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![Figure 3](image3.png)

Table 2: In vitro potency of test ligands in mimicking estrogenic actions

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![Figure 4](image4.png)

Figure 4: Discussion

We have recently reported our synthetic strategy in the preparation of a series of 17α-(Substituted-phenyl)vinyl Estradiols. Although the yields were not optimized, we demonstrated the feasibility of preparing these agents by a variety of synthetic and solid phase methods. In addition, we have synthesized a series of test ligands with varying affinities and have determined their in vitro and in vivo potency.

Summary:

The 17α-(Substituted-phenyl)vinyl Estradiols are a novel class of estrogen ligands that exhibit potent estrogenic activity in vitro. The use of these compounds as potential modulators of the estrogen receptor is a promising area of research. Further work is needed to explore the potential of these compounds as modulators of the estrogen receptor.

References:


Acknowledgments:

This work has been supported by grants from the US NIH (ROI-CA-58184 to RRG), UNIV (171-0-1533 and 171-000-30), and the Japanese Ministry of Education, Science, and Culture (B65). Support for the Molecular Modeling Center was provided by NSF (CHE-9759462).
The Mitsunobu Reaction: A versatile synthetic and educational tool

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Objectives:
These are the objectives for the undergraduate research project undertaken by our group:
- The students should gain an understanding of the Mitsunobu reaction, its mechanisms, and its applications as a versatile synthetic tool.
- The students should become proficient in planning and executing synthetic experiments, including the use of solvents and reagents.
- The students should gain an appreciation for the historical context and development of the Mitsunobu reaction.

Introduction:
One of the major areas of research in our group involves the development of synthetic methods for the construction of nitrogen-containing heterocycles. The nitrogen-oxygen (NO) moiety is a common structural feature in many natural products and pharmaceuticals, and a versatile building block in organic synthesis. Our group has developed a protocol for the Mitsunobu reaction, which is a classic example of a high-yield, high-selectivity reaction for the construction of nitrogen-containing heterocycles.

The Mitsunobu Reaction:
The reaction involves the treatment of an activated ester with pyridine and diisopropylethylamine to form the corresponding amine. The protocol is simple and efficient, and can be used for the synthesis of a wide range of nitrogen-containing heterocycles.

Results and Discussion:
The project provided the opportunity to examine a synthetically useful sequence. Although the Mitsunobu is essentially a one-step reaction, it proceeds via several intermediates. The mechanism involved in the conversion of the starting material to the product is complex, involving the formation of a tetrameric intermediate, which then rearranges to give the final product. The efficiency of the reaction is high, and the yields are reproducible.

The Mitsunobu Reaction can be performed in a variety of solvents, including polar aprotic solvents such as DMF and DMSO. The reaction is also tolerant of a wide range of functional groups, including esters, ethers, and amides.

Approaches to the Problem:
The reaction was designed to be carried out under mild conditions, using a catalytic amount of triethylamine and diisopropylethylamine. The reaction was carried out at room temperature, and the reaction time was 2-4 hours. The reaction was monitored by thin-layer chromatography, and the product was isolated by column chromatography. The purity of the product was checked by 1H NMR and 13C NMR spectroscopy.

In conclusion, the Mitsunobu reaction is a powerful synthetic tool for the construction of nitrogen-containing heterocycles. The reaction is simple, efficient, and tolerant of a wide range of functional groups. The protocol is suitable for both synthetic and educational applications.

Figure 1: An illustration of the Mitsunobu reaction

Figure 2: A comparison of the yields obtained using the Mitsunobu reaction and other methods

Figure 3: A schematic representation of the reaction mechanism

Figure 4: The structure of the compound synthesized using the Mitsunobu reaction

Figure 5: A photograph of the synthesized compound
Rachel Gershman¹, Robert N. Hanson¹, Eugene R. DeSombre², and Alun Hughes². (1) Department of Chemistry, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, rgershma@lynx.neu.edu (2) Ben May Institute for Cancer Research, University of Chicago, 60637.

Abstract
As part of our program to develop novel selective estrogen receptor modulators (SERMs), we chose to prepare and evaluate a series of 4-substituted-3,5-diaryl-isoxazoles. Based upon ongoing projects, we elected an approach by which the target compounds 1 could be obtained via palladium-catalyzed coupling reactions. In this preliminary study, Sonogashira and Stille reactions with 4-iodoisoxazole 2 were investigated to introduce alkynyl groups. The Suzuki reaction was examined by coupling 2 with phenylethenylboronic acids and by the reverse route of coupling isoxazole ethynylboronic acid 3 with aryl iodides. Synthetic and biological results will be discussed.

Introduction
Breast cancer is the most common cancer and the second-leading cause of cancer-related deaths in women.
- Tamoxifen, the most commonly used drug for treatment of breast cancer, is a selective estrogen receptor modulator (SERMs) that acts as an antagonist in the breast, blocking estradiol and stopping tumor growth.
- However, tamoxifen acts as an agonist in the uterus, causing increased risk of endometrial cancer.

- Raloxifene, currently used for the prevention of osteoporosis, shows promising agonist/antagonist activity without stimulation in the uterus.
- Tetrasubstituted pyrazoles and triphenylisoxazoles that are currently being studied also show promising results.

Goals
- Synthesize novel 4-E-2(4-Rphenyl)-3,5-diaryl-isoxazoles 1 via palladium-catalyzed coupling reactions.
- Investigate the synthesis by two approaches (Scheme 1).
- Demonstrate the feasibility of these synthetic routes and the potential for future development of combinatorial libraries.

Chemistry
- Suzuki coupling of 4-iodoisoxazole 2 with phenylboronic acids afforded products 6a-d in high yield.
- Sonogashira and Stille couplings of 2 gave low yields of 4-ethynylisoxazole 4.
- Hydroboration/Suzuki coupling gave moderate conversion to 6a-d.

Biology
- Dihydroxy compounds 1a-d exhibit modest binding affinity to ERα.
- However, compounds 1a-d are highly selective for ERα over ERβ.

Conclusion
- Route b (Scheme 1) proved to be more difficult than expected; however, route a is limited by the number of commercially available phenylboronic acids.
- Nevertheless, this study demonstrates that 4-substituted-3,5-diaryl-isoxazoles are accessible by the two synthetic routes featuring palladium-catalyzed coupling reactions.
- Although compounds 1a-d show modest binding affinity, they show promising selectivity for ERα.
- Future work includes further investigation of the hydroboration/Suzuki coupling sequence to generate a larger series of derivatives for optimization.

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