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Award Number: W81XWH-04-1-0544

TITLE: Development of Novel Technetium-99m-Labeled Steroids as
Estrogen-Responsive Breast Cancer Imaging Agents

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REPORT DATE: June 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20051018 181

REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 01-06-2005		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 15 May 2004 - 14 May 2005	
4. TITLE AND SUBTITLE Development of Novel Technetium-99m-Labeled Steroids as Estrogen-Responsive Breast Cancer Imaging Agents				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-1-0544	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Robert N. Hanson, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Northeastern University Boston, MA 02115				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT – SEE ATTACHED PAGE					
15. SUBJECT TERMS synthesis, rhenium-technetium complexes, imaging agents					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Abstract

The overall objective of this project is the development of new Tc-99m-labeled steroids that can be used for single photon imaging of estrogen responsive breast cancer. The technical aims for the initial period involved preparation and characterization of an initial series of functionalized estradiols, their evaluation as ligands for the estrogen receptor, and initial conversion to the corresponding rhenium tricarbonyl derivatives. We have successfully undertaken these specific aims. We have prepared several pyridyl and histidinyl containing estradiols using Stille and Suzuki coupling methods, and characterized them using NMR and elemental analysis or MS. The appropriate biological assays- ER-alpha and ER-beta ligand binding domain competitive binding assays, and induction of alkaline phosphatase in Ishikawa cells- have been established and applied to several compounds in the initial series. Experiments evaluating the coordination of rhenium carbonyl reagents with pyridyl, bipyridyl and histinyl species are in progress. Aims for the second year include completion of these initial goals and evaluation in vitro and in vivo of the rhenium tricarbonyl-estrogen complexes.

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1. Introduction

This proposal focuses on the design, preparation and evaluation of new Tc-99m-labeled steroids for the imaging of estrogen-responsive breast cancer. Based upon our previous studies we have designed a series of estradiol derivatives capable of binding Tc-99m, the radionuclide of choice for single photon emission computed tomography (SPECT) in nuclear medicine. These compounds can be obtained using Pd(0) catalyzed coupling reactions between an appropriately substituted estradiol and the corresponding chelating group. Biological evaluation of the new materials includes competitive binding assays to determine affinity as well as cellular assays to examine efficacy and selectivity. Subsequent coordination with rhenium carbonyl, a nonradioactive correlate of Tc-99m, will provide the basis for subsequent structural modifications and selection of a potential radioactive agent for in vivo imaging/tissue distribution studies.

2. Body

The specific aims for the first year included the synthesis of the initial series of estradiol derivatives, development of the bioassay systems and preliminary evaluation, and preparation of the first rhenium tricarbonyl derivatives.

2.A. Synthesis of initial series.

We have employed two strategies to obtain the target compounds, both proceeding via the stannylvinyl estradiol intermediate. In the first strategy, we perform the Pd(0) coupling (Stille reaction) with the corresponding aryl halide. In this case we have used the 3-, and 4-bromopyridines. We have prepared the 2-bromobipyridine but have not done the coupling. Alternatively, we converted the stannylvinyl estradiol to the iodovinyl derivative which was then coupled with the corresponding arylboronic acid (Suzuki reaction). This was done with 3- and 4-pyridine boronic acid. Both methods are successful in generating the final compounds but the latter method uses less toxic materials, proceeds under milder conditions and has a somewhat easier isolation procedure. We are evaluating whether the extra step results in a significantly improved overall yield.

We have also prepared our initial histidinyl estradiol. Bis-iodobenzoylation of histidine methyl ester followed by selective deprotection gave the N-iodobenzoyl-histidine methyl ester which was coupled (Stille reaction) to the stannylvinyl estradiol in modest yields. The new product has been characterized and subsequently hydrolyzed to the free acid in preparation for coordination with rhenium tricarbonyl.

2.B. Development of bioassays.

We have generated the assay system for determining the relative binding affinity (RBA) for each of the new estradiol derivatives. We will be using both the ER-alpha and ER-beta ligand binding domains to look at how well the compounds compete for the ligand binding site. This assay will also determine the relative selectivity of the compounds for one receptor subtype versus the other because ER-alpha is associated more strongly with breast cancer than ER-beta. Another important assay is the induction of alkaline phosphatase (AlkP) in Ishikawa cells. This is a functional assay that looks at how well the compounds are able to function as agonists or block the action of an agonist such as estradiol. We have

currently performed these studies on a series of structurally related compounds (previously prepared in our laboratory) to establish the conditions and set a "baseline" for binding and stimulatory activity. Evaluation of the specific compounds prepared in 2A. is in progress.

2.C. Preparation of rhenium tricarbonyl complexes with estradiol derivatives.

We have started the studies involving the preparation of the rhenium tricarbonyl complexes. We have proposed three different types- mono-pyridyl, bipyridyl, and histidinyl estradiols. Our initial work has focused on the chemistry related to the coordination of the rhenium carbonyl species with simple pyridines, bipyridines and the iodobenzoylated histidine. Coordination with the first two has been completed and the third is in progress. Extension to the corresponding estradiol derivatives will be undertaken when all of the compounds are available.

3. Key research accomplishments

- Synthesis of pyridyl-, and N-iodobenzoylhistidinyl estradiols in good yields
- Development of alternate routes to ligand preparation
- Establishment of bioassays for binding and efficacy
- Development of coordination methods for rhenium carbonyl species

4. Reportable outcomes

The results of this project were reported at the ERA of HOPE meeting in Philadelphia, PA on June 8-11,2005. Poster 16-12.

5. Conclusions

The project is currently proceeding as planned and on schedule. Tasks 1-3 are scheduled to be completed by the end of Year 02 with possible entry into Task 4 underway in that time frame. Identity of target compounds for Task 4 will depend upon the results obtained from Tasks 2 and 3, but the synthetic methods will already be established.

6. References

None.

7. Appendices

None.