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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Estradiol works at the level of the genetic material of the breast epithelial cells to control a wide range of genes that determine how fast the cell will grow. Breast cancer cells often remain sensitive to estradiol subsequent to becoming cancer cells. Type I 17β-hydroxysteroid dehydrogenase (HSD) is the enzyme responsible for reducing the hormone estrone to estradiol in the epithelial cells of the breast. In many cases of breast cancer, elevated quantities of HSD have been observed associating it with abnormal cell proliferation. It has therefore become our task to try and inhibit HSD's catalytic function. We have discovered that dihydroxynaphthoic acids inhibit dehydrogenase enzymes, and we also know how to design variations among this class of inhibitors. With intentions of finding a new cancer therapeutic, it has been our goal to utilize structure-based drug design and molecular modeling to develop selective inhibitors of human HSD and to test these for activity against human breast cancer cells grown in culture.

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Introduction:

Hydroxysteroid dehydrogenases (HSD) play an essential role in the biosynthesis and degradation of all steroid hormones. 17β-HSDs are a subclass of HSD isoenzymes that specifically participate in the final steps of the synthesis of estrogens and androgens. Human type 1 17β-HSD, also known as 17β-estradiol dehydrogenase, catalyzes the reduction of estrone to 17β-estradiol, the biologically active estrogen involved in the development of human breast cancer. Since 17β-estradiol controls cell proliferation via ligand occupancy of the nuclear estrogen receptor, 17β-HSD and its associated activation of this steroid, has become the key enzyme for cellular proliferation. Type 1 17β-HSD can, therefore, be viewed as a molecular switch for cell proliferation. As this enzyme is present in elevated concentrations of hormone-sensitive breast cancer cell lines, a compelling case can be made for the development of selective inhibitors of 17β-HSD as an approach to the design of new therapeutics in the treatment of breast cancer. We have recently developed a new class of dehydrogenase inhibitors that are targeted at the NAD(P)/NAD(P)H binding sites of dehydrogenases. Collectively, these binding sites contain a specific three dimensional array of parallel beta strands and associated alpha helices that are known as the dinucleotide or Rossmann fold. Surprisingly, these inhibitors exhibit selectivity for different dehydrogenases based on slight variants in the amino acid moieties of their Rossmann folds. The goal of this project is to develop selective inhibitors of Type 1 17β-HSD as "lead compounds" for structure-based drug design using the novel concept of targeting the Rossmann fold for inhibiton.

Body:

In the statement of work, the following tasks were proposed:

Task 1

The development of synthetic schemes for preparation of mono- and dihydroxynaphthoic acids as potential inhibitors of 17β-HSD-1 (months 1-36, an ongoing activity). New synthetic methodologies will be explored focusing on synthon development towards more efficient convergent syntheses. Using an optimized convergent synthesis, a higher number of variant substituted synthons can be made and used in the same time frame. In this way more target compounds can be made in the same period, and with higher overall yields.

Task 2

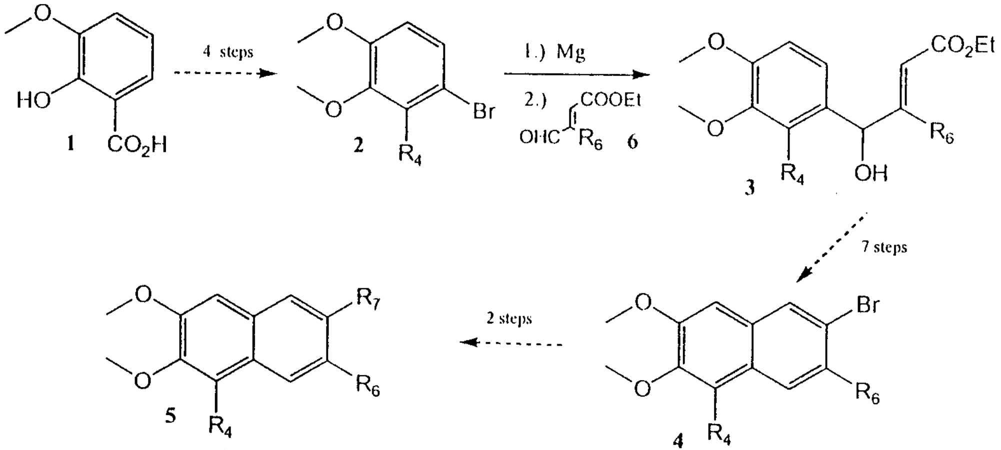
The development of combinatorial libraries (months 1-36, an ongoing activity). New and old synthetic methodologies will be combined for combinatorial syntheses of specific libraries for higher thruput activity screening against different dehydogenases. These methods lend themselves to parallel synthesis particularly well.

Task 3

The development of Pan-Active-Site inhibitors, directed by molecular modeling and kinetic results (months 1-36, an ongoing activity). Information from modeling and screening of libraries will be used in conjunction to probe the mechanism of inhibition as well as to design next generation inhibitors.

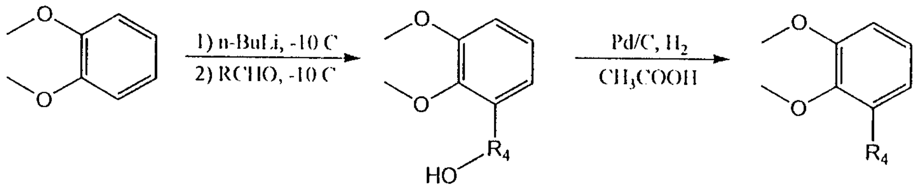
Recent developments in our synthetic scheme have addressed not only the convergence and efficiency our synthetic scheme but have also introduced steps that allow for more diversity in the four, six and seven positions as well[1-4]. Some of the first dihydroxynaphthoic acids created by our original synthetic scheme were limited to small alkyl groups such as methyl, ethyl, n-propyl and isopropyl in the four position[5-8]. More recent developments in this scheme have allowed us the luxury of introducing a wide variety of new alkyl groups in the four position while lowering our costs. Previous methods involved the use of 3-methoxysalicylic acid as a starting material (scheme 1).

Scheme 1



This reagent was not only costly but was limited in its potential diversity due to the acid group. New methods involve the use of veratrole as a starting material. Veratrole not only costs less, but may be easily recovered from completed reactions allowing for quantative yields. Moreover, the reaction of veratrole with n-butyllithium and aldehydes seems to be limitless in its potential to impart different alkyl and aralkyl groups in the four position (scheme 2).

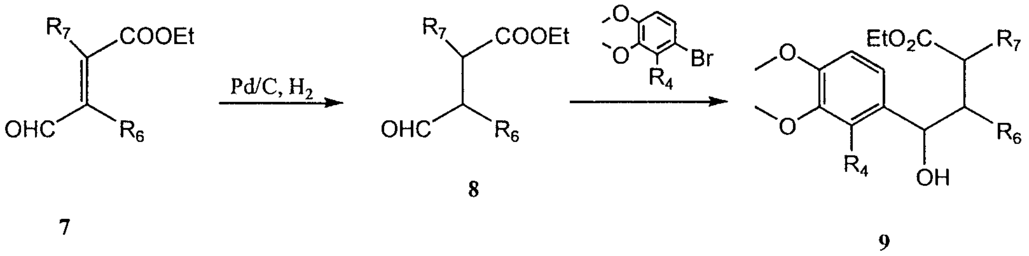
Scheme 2



Additionally, computer modeling has suggested that large lipophilic groups in the four position also lend to a greater potential for developing Pan-active inhibitors. Scheme 2 allows for the introduction of a larger lipophilic center in the four position and an inhibitor with an n-Butyl group is currently being synthesized.

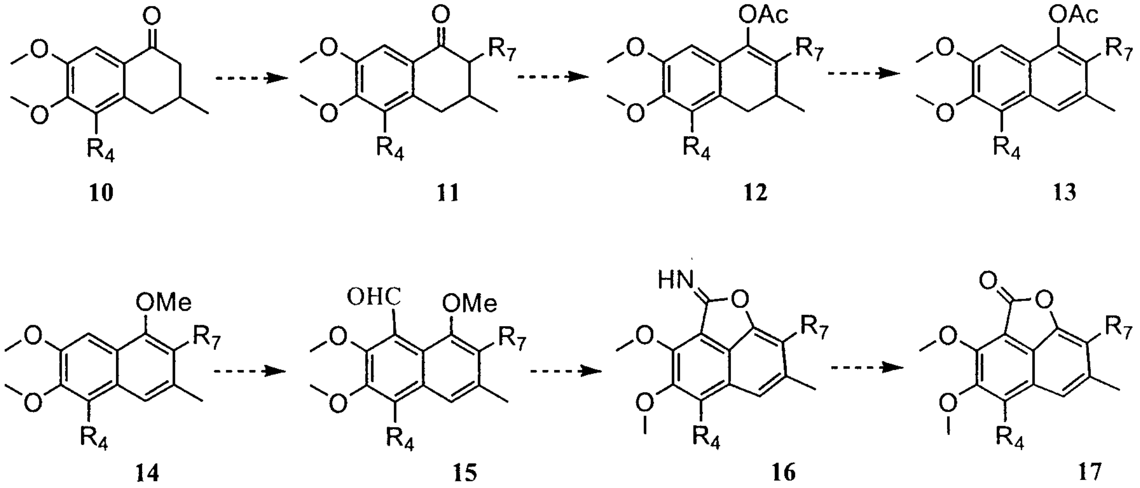
The past year has also seen some new developments with respect to synthon chemistry. Previous methods relied on utilizing this compound in its unsaturated form (compound **6**) with a methyl group in the three position. As a result, subsequent reactions involved the hydrogenation of the alkene and hydrogenolysis of the alcohol functionality resulting from the synthon addition (compound **9**). The reduction of these two functional groups typically rendered lower yields in this step, and action has since been taken to alleviate this problem. Currently we are exploring the use of the synthon in its saturated form (**8**) rendering the alkene reduction obsolete (scheme 3). Additionally, we have also explored the use of (**6**) without the methyl in the three position. As these reactions have shown themselves to be successful, we have now been introduced to the possibility of changing the alkyl substituents at the six and seven positions of the naphthalene ring by altering the three and two subsituents on the synthon respectively (scheme 3).

Scheme 3



Mike Brown and I have computer modeled 17β-HSD with naphthoic acid inhibitors resulting in a conclusion that the introduction of a lactone functionality (scheme 4) in place of the acid group could result in more potent inhibitors. Accomplishing this requires the alkylation of the tetralone intermediate (**10**) of the synthetic scheme using potassium hydride and alkyl halides. The substituted tetralones were treated with isopropenyl acetate and p-toluenesulfonic acid to form enol acetates (**12**). The enol acetates were then treated with DDQ to yield phenolic acetates (**13**) which may then be hydrolyzed to the corresponding phenols and methylated to form trimethoxynaphthalenes (**14**). The procedure used to make the 7-substituted hemigossypols will be similar to those used to make 7-substituted deoxyhemigossylic acids and 7-substituted deoxyhemigossypols. Iminolactone and derivatives of compound (**15**) will be made by the same procedures used in the laboratory to make the corresponding derivatives of gossypol[4].

Scheme 4



These more convergent and efficient schemes, backed by molecular modeling of our compounds with 17β-HSD, have vastly improved our potential to quickly synthesize a battery of compounds leading to more potent dehydrogenase inhibitors. As such, we are much closer to establishing a combinatorial library of naphthoic acids that not only promise to be more effective in their inhibition dehyrdrogenase enzymes, but also promise to give us more insight into the behavior of the inhibitors with respect to their four, six and seven position substituents. Increasingly, these methods will lend themselves to establishing a more parallel synthesis for any inhibitors made in the future and will help to probe the mechanism associated with reduction at the Rossmann fold.

Key research accomplishments:

- Synthetic scheme has been made more convergent and efficient
- Synthetic scheme has added potential for diversity of R groups in four, six and seven positions of naphthalene ring
- Introduction of R groups to positions previously unavailable
- Use of molecular modeling to begin the synthesis compounds with computer substantiated R groups
- The completed synthesis of several inhibitors as a preempt to a combinatorial library for parallel synthesis

Conclusion:

The past year has shown exciting new developments with respect to the synthetic process used to make our compounds. Already, we have seen our synthesis become more efficient and diverse; opening new possibilities with respect to the size, electronic behavior and position of new R groups that were previously thought to be out of the scope of our scheme. At the moment, additional work is being done to attempt to eliminate some of the harsher reagents involved in our synthesis. Should these reactions prove effective, more chemically sensitive R groups may be attempted. Additionally, we have seen an increase of the cost and time efficiency of our synthesis which has allowed us to explore new methods to further converge and facilitate the making of previous and new inhibitors. Computer molecular modeling has given us even more insight into how our inhibitors are behaving with respect to their position and coordination within the Rossmann fold. All of these factors are leading us to find an ever increasing amount of possible variations for our inhibitors widening the potential for finding an effective Pan-active inhibitor.

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