SBIR PHASE I REPORT

Title: TARGETED MOLECULAR RADIOTHERAPY OF ESTROGEN RECEPTOR POSTIVE TUMORS

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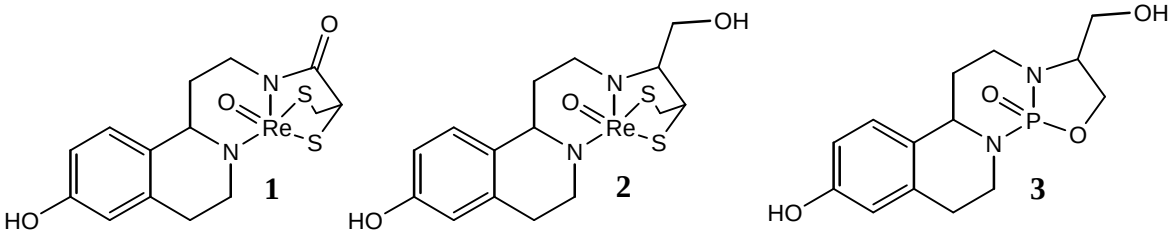
The overall objectives of the proposal were to develop estrogen receptor (ER) binding small molecule radiopharmaceuticals for targeted radiotherapy of ER positive (ER+) tumors. In particular, this proposal focused on embedding a ^{186,188}Re or a ^{32}P radionuclide into an estrogen steroidal framework by isosteric substitution such that the resulting structure is topologically similar to the estrogen (estrogen mimic). The estrogen mimic molecules expected to bind to the ER and exhibit biodistribution akin to that of native estrogen due to structural mimicry. It is anticipated that the ^{186,188}Re- or a ^{32}P-containing estrogen mimics will be useful for targeted molecular radiotherapy of ER+ tumors. .

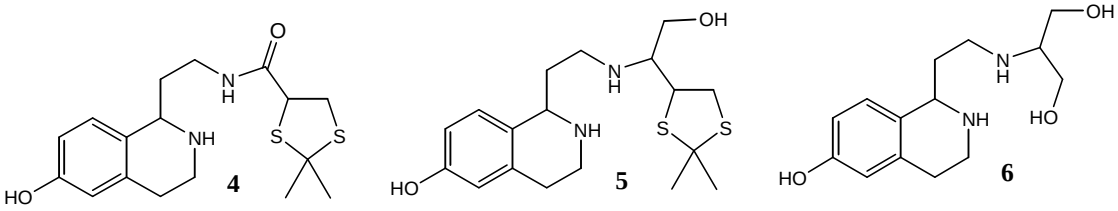
It is well established that the *in vivo* target tissue uptake of estrogen like steroidal molecules is related to the binding of the steroids to sex hormone binding globulin (SHBG). SHBG is important in the uptake of estrogens and testosterone in target tissues by SHBG receptors on the cell surface. However, hitherto the design of estrogen like small molecule radiopharmaceuticals was focused on optimizing ER binding characteristics without emphasis on SHBG binding properties. Consequently, even the molecules with good ER affinity *in vitro*, performed poorly in biodistribution studies. Based on molecular modeling studies the proposal focused on developing estrogen mimics **1-3** which were topologically similar to native estrogens, and form hydrogen bonds in ER and SHBG in the same manner as those of native estrogens.

To this end the technical objectives of the proposal focused on synthesizing the rhenium-estrone and estradiol mimics **1** and **2** respectively, and phosphorous estradiol mimic **3**



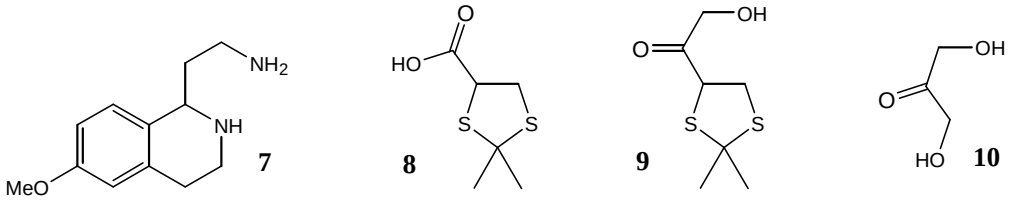
and to assess their stability and in vitro binding characteristics to ER and SHBG.

Ligands for Synthesis:



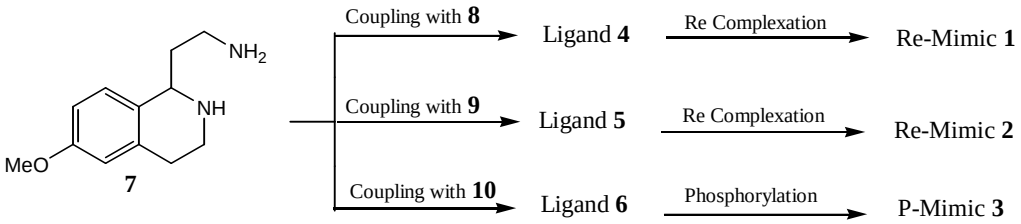
Isosteric substitution at position 14 in estradiol with rhenium or phosphorous requires the synthesis of a molecular framework that contains amino, amido, mercapto, and hydroxyl donors capable of forming N_2S_2 metal complexes or N_2O phosphoramidates having a square-pyramidal or a tetrahedral geometry, respectively. In the molecular design, the heterocycle constitute A and B rings of steroid skeletal framework, and the metal ion tether the side chain in such a manner as to form the C-D ring portions.

Retrosynthetic analysis of **4-6** gives four key intermediates: tetrahydroisoquinoine **7**, the thioketals **8** and **9**, and the diol **10**.



These key intermediate were anticipated to be used in the preparation of rhenium and phosphorous estrogen mimics **1**, **2** and **3** as sketched in Scheme 1. The 3-MeO group

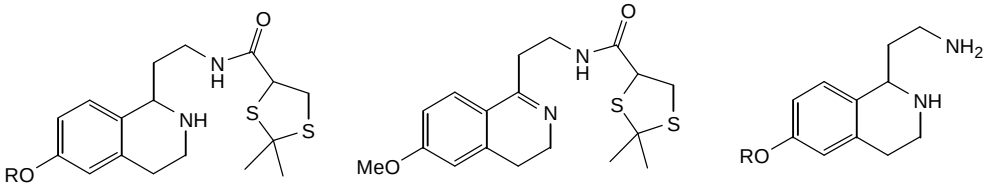
Scheme 1



of the intermediate **7** will be converted to 3-OH group during one of the steps in Scheme **1.**

The initial efforts were directed at the synthesis and evaluation of the N_2S_2 mimic **1** or 2. Since the synthesis of common intermediate **7** is essential for the whole process most of the efforts were focused on the synthesis of this fragment.

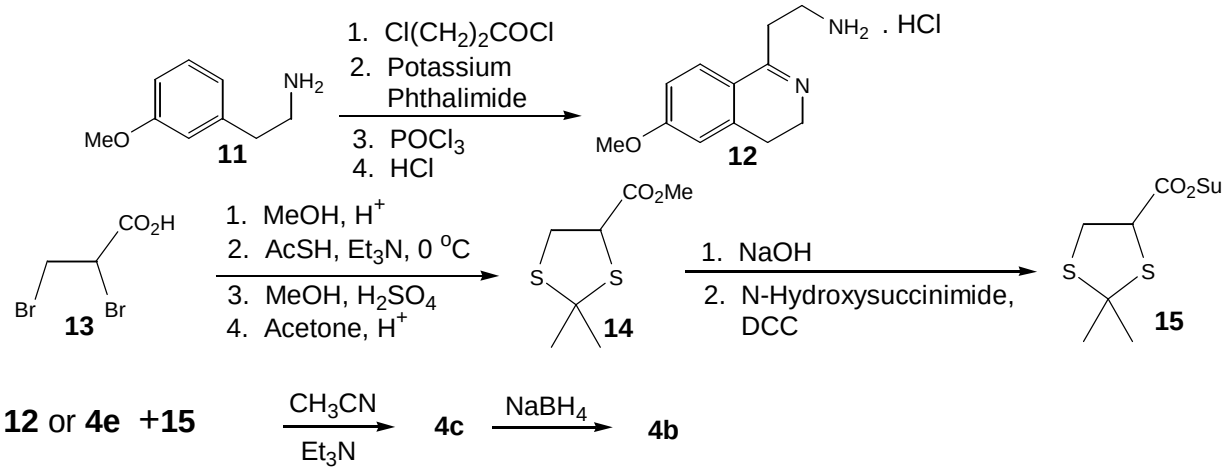
Ligand Synthesis: As will be discussed below, the N_2S_2 ligands **4 a**-**f** have been prepared. The initial synthetic strategy for **4 a-b** involved six different approaches using



4a: R = -H **4b**: R = -Me **4c**: R = -Me **4d**: R = -Bn **4e**: R = -Me **4f**: R = -Bn

Bischler-Napieralski and Pictet-Spengler cyclizations. Synthesis of ligand **4a-d** and the intermediates **4e-f** and the active ester **15** longer time than anticipated due to various technical difficulties. After considerable effort, ligands **4 a-b** and

Scheme 2



4 c-d were prepared in twelve steps as outlined in Scheme **2**. The aminoimine **12** was prepared from commercially available 3-methoxyphenylethylamine in four steps. Preparation of the thioketal active ester **15** proved to be considerably more difficult than had been anticipated. It was finally prepared in six steps from 2,3-dibromopropionic acid. The alkylation of 2,3-dibromopropionate with thiolacetic acid must be carried out at cold temperature, and the saponification of the corresponding bis(thioacetate) must be carried out under acidic conditions over a period of 48 hours. The active ester **15** was condensed with the aminoimine **12** to give the imino ligand **4c-d**, which was then reduced with sodium borohydride to give the desired amino ligand **4b** as a roughly equal mixture of two diastereomers.

The synthetic method developed for ligand **4b** was further modified for the synthesis of ligand **4a** and it was completed. Ligand **4a** was synthesized in a similar fashion starting from 3-benzyloxy-phenylethylamine. The amine **4e** was prepared in two steps from 3-benzyloxybenzaldehyde by the published method. Reaction of **4f** with chlorpropionyl chloride, alkylation of the chloride with potassium phthalimide, and Bischler-Napieralski cyclization of the phthalimide gave the desired imine **4d**. The ligand **4a** was obtained roughly equal mixture of two diastereomers as **4b** and Tc/Re metal complexation of ligands **4b** and **4a** are in progress to evaluate their ER and SHBG binding characteristics.

The synthesis of intermediates **9** and **10** are continuing to obtain ligands **5-6** for estrogen mimics **2** and **3**. The initial determination of ER and SHBG binding studies with the diasteroemeric complexes of **4a** will provide some insight into the usefulness of the estrogen mimics **1** as well as the ongoing work on the preparation of estrogen mimics **2** and **3.**