**DOE SBIR PHASE I FINAL TECHNICAL REPORT**

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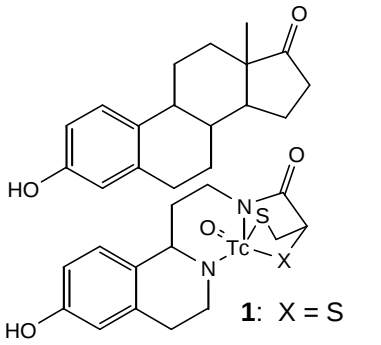
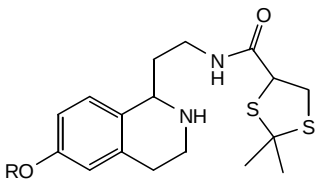
**Title:** Novel Metal Ion Based Estrogen Mimics for Molecular Imaging

The overall objective of the SBIR Phase I proposal is to prepare and evaluate a new class of $^{99m}$Tc or $^{94m}$Tc containing estrogen-like small molecules ('estrogen mimics') for SPECT or PET molecular imaging of estrogen receptor positive (ER+) tumors. In this approach, the metal ion is integrated into the estrone skeleton by isosteric substitution of a carbon atom in the steroidal structure to give new class of mimics that are topologically similar to the native estrogen (Fig. 1). Although both $N_2S_2$ and $N_3S$ mimics **1** and **2** were considered as target structures, molecular modeling study revealed that the presence of the acetyl group at position-15 in the $N_3S$ mimic **2** causes steric hinderance toward binding of **2** to SHBG. Therefore, initial efforts were directed at the synthesis and evaluation of the $N_2S_2$ mimic **1**.

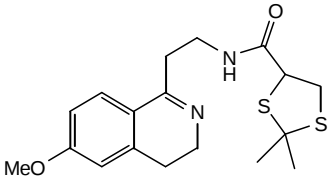


**1**: X = S
**2**: X = NAc
Fig. 1

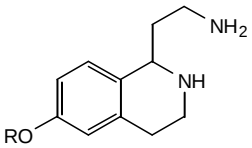
***Ligand Synthesis***: As will be discussed below, the $N_2S_2$ ligands **3b** and **4** have been prepared, and the preparation of **3a** nearing completion. Retrosynthetic analysis of ligands **3** and **4** yielded the tetrahydroisoquinoline fragments **4** and **5**, and the dimercaptopropionate fragment **6**. The initial synthetic strategy for **3a,b** involved six different approaches using



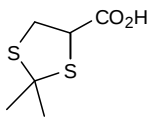
**3a**: R = -H **3b**: R = -Me



**4a**: R = -Me **4b**: R = -Bn



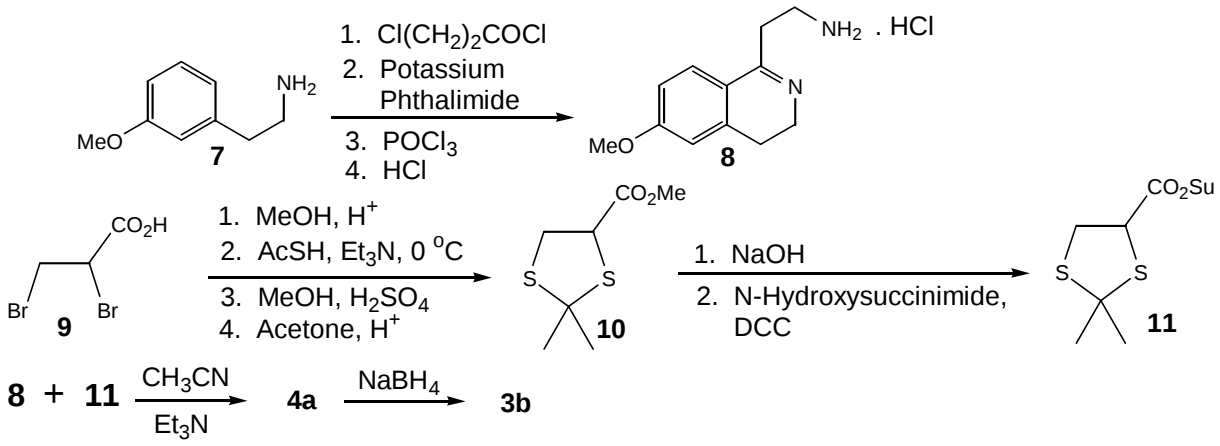
**5a**: R = -Me **5b**: R = -Bn



**6**

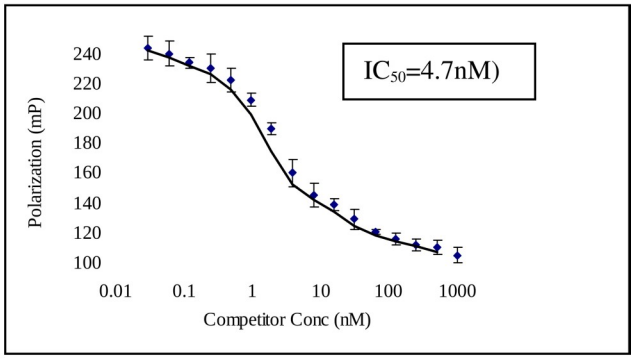
Bischler-Napieralski and Pictet-Spengler cyclizations. After considerable effort, ligands **3b** and **4** were prepared in twelve steps as outlined in Scheme 1. The aminoimine **8** was prepared from

**Scheme 1**

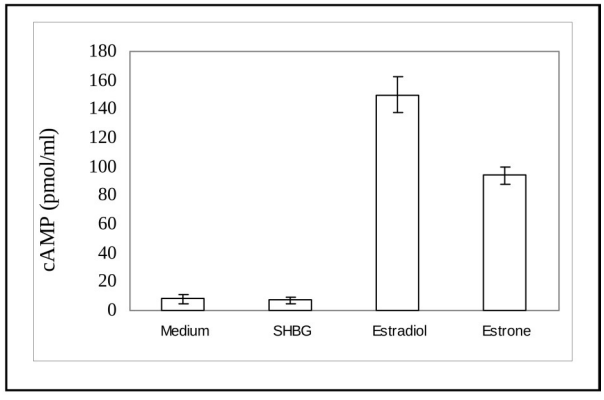


commercially available 3-methoxyphenylethylamine in four steps. Preparation of the thioketal active ester **11** proved to be considerably more difficult than had been anticipated. It was finally prepared in six steps from 2,3-dibromopropionic acid. The alkylation of 2,3-dibromopropionate with thiolacetic 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 **11** was condensed with the aminoimine **8** to give the imino ligand **4**, which was then reduced with sodium borohydride to give the desired amino ligand **3b** as a roughly equal mixture of two diastereomers. The synthesis of ligand **3a** is nearing completion, and is being synthesized in a similar fashion starting from 3-benzyloxy-phenylethylamine. The amine **5b** was prepared in two steps from 3-benzyloxybenzaldehyde by the published method. Reaction of **5b** with chlorpropionyl chloride, alkylation of the chloride with potassium phthalimide, and Bischler-Napieralski cyclization of the phthalimide gave the desired imine **4b**. Tc/Re metal complexation of ligands **3b** and **4a** are in progress.

***ER Binding Assay*:** Baculovirus expressed ER¬λ was mixed with fixed concentration (40 nM) of flurochrome labeled estradiol (ES2) (Flurmone-ES2) to form ER/flurmoneES2 complex. This complex was then added to varying concentration of (0.01 nM to 1μM) of 17β⊏-estradiol as competitors in separate wells. The flurmone-ES2 has high polarizing value when it is bound to the ER and low polarizing value in its free form. Increasing the concentration of competitor displaces the ER bound Flurmone-ES2 resulting in low polarizing value. For establishment of assay, 17β⊏-estradiol was used as a model competitor and as demonstrated in the adjacent figure increasing the concentration of competitor estradiol results in the displacement of flurmone-ES2 from ER. The polarizing values of each concentration of the competitor were plotted against the competitor concentration and the $IC_{50}$ value was determined from the curve. As demonstrated in the figure, the calculated $IC_{50}$ of estradiol from our assay (4.7 nM) is similar to that of the reported values in the literature (4-6nM). This assay procedure will be used to determine the ER binding of estrogen mimic **1**.




***Assay to determine the binding characteristics of estrogen mimics to cell membrane bound SHBG:*** We have established the assay in A laboratory using MCF-7 breast carcinoma cells. MCF-7 cells (5 X $10^6$) plated in serum free medium 18 hours before the experiment in 6-well plates were washed and the SHBG receptors on the cell surface are saturated with sufficient (50ug / ml) concentration of free SHBG and allowed to incubate for 30 min at $37^0$ C. The cells were washed with serum free medium and 100nM of estradiol or estrone were added and intracellular cAMP levels were measured at 15-20 min. later using commercially available ELISA kits (R & D Systems, Inc., MN). The results of the experiment described above using estrone and estradiol are illustrated in Fig. A above. The response of the steroid to induce cAMP upon binding to membrane bound SHBG was dose dependent as demonstrated in Fig. B. The



assay described above will be used to determine whether the estrogen mimics induce signaling response like an agonist. Further increase in cAMP concentration following each estrogen mimic addition will indicate that the estrogen mimic is binding in same configuration in SHBG as that of estrone or estradiol, since DHT that binds in different orientation does not induce cAMP in androgen receptor positive prostate cells. The SHBG binding studies will be carried out in manner similar to that of ER binding assay and are in progress.

B