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Separation of stereoisomers of 7-oxa-bicyclo[2.2.1]heptene sulfonate (OBHS), a Selective Estrogen Receptor Modulator (SERM), via chiral stationary phases using SFC/UV and SFC/MS

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ABSTRACT

The enantiomeric separation of a racemate of 7-oxa-bicyclo[2.2.1]heptene sulfonate (OBHS) derivatives, a Selective Estrogen Receptor Modulator (SERM), was obtained using supercritical fluid chromatography in tandem with UV and mass spectrometry (SFC/UV and SFC/MS, respectively). Supercritical CO_2 modified with methanol or isopropyl alcohol was used with isopropylamine (IPAm), trimethylamine (TEA), or trifluoroacetic acid (TFA) as an additive to obtain the enantiomers separations. Both Chiralpak IC and IA were evaluated for the separation of enantiomers. Results showed enantiomers separation can be achieved in less than 5 min with a resolution greater than 1 and 0.9, respectively, for the different OBHS derivatives (compounds A and B) using supercritical CO_2 modified with 40% isopropyl alcohol containing 0.25% IPAm and IC column applying isocratic conditions. Similar conditions were used with the semi-preparative Chiralpak IC column to isolate more than 50 mg of each enantiomer. SFC/MS and SFC/UV results showed pure enantiomers were isolated. Method development via SFC was much simpler than those reported in the literature using HPLC.

1. Introduction

Estrogen receptor (ER), is a steroid hormone receptor, essential for the normal reproductive and cellular functions in both humans and animals [1]. About 70% breast cancers have been shown to be ER positive and display responsiveness to endocrine therapies with varying success [2]. It has been demonstrated that estrogen receptor (ER) is capable of binding and responding to different steroidal and non-steroidal ligands, with low nanomolar to picomolar potency. Several ligands have been developed that can modulate ER expression and function in a tissue-specific manner by being stimulatory in one and inhibitory in another tissue (thus called Selective Estrogen Receptor Modulators, SERMs) or that can both inhibit and degrade ER (Selective Estrogen Receptor Degraders). These ligands generally have flat aromatic framework incorporating a stilbene core in some fashion and appended with a basic heterocyclic chain or an acrylic acid core that interfere with the critical folding and binding of helix 12, which is necessary for recruiting coactivators to activation function 2 (AF2 pocket) during the transcriptional process [4]. However, most of these clinical or experimental therapeutics suffer from unwanted side effects, formulation issues, and most importantly, eventual emergence of cancer resistance after prolonged treatment [3]. These anti-estrogens also show weaker binding to the clinically relevant ER mutants, Y537S and D538G. Thus, our interest lies in the development of three-dimensional sp3-rich anti-estrogenic that could better accommodate into the hydrophobic pockets, and be active against both wild-type and mutant forms of ER.

After the initial discovery and report of a non-steroidal three-dimensional ligand core - 7-oxa-bicyclo[2.2.1]heptene sulfonate (OBHS) (Fig. 1A) by the Katzenellenbogen group [4], we sought to further develop this agent for modulating helix 8/11 axis of ER that further interferes with helix 12 (thus, an indirect antagonist). The 7-oxa-bicyclo [2.2.1]heptene sulfonate (OBHS) bicyclic system would be an appropriate promising new class of drugs for the estrogen receptors. The Josan group at Virginia Tech discovered several analogs of OBHS substituted on the para position of the phenylsulfonate group that showed high binding affinity and selectivity to ERα over ERβ [5] isoform, and that showed marked antiproliferative and anti-inflammatory activity (manuscript in preparation). The OBHS analogs were obtained from the Diels-Alder reaction of 3,4-disubstituted furan and a derivative of vinyl phenyl sulfonate, giving rise to exo and endo diastereomers. The desired exo diastereomer was separated from endo via flash

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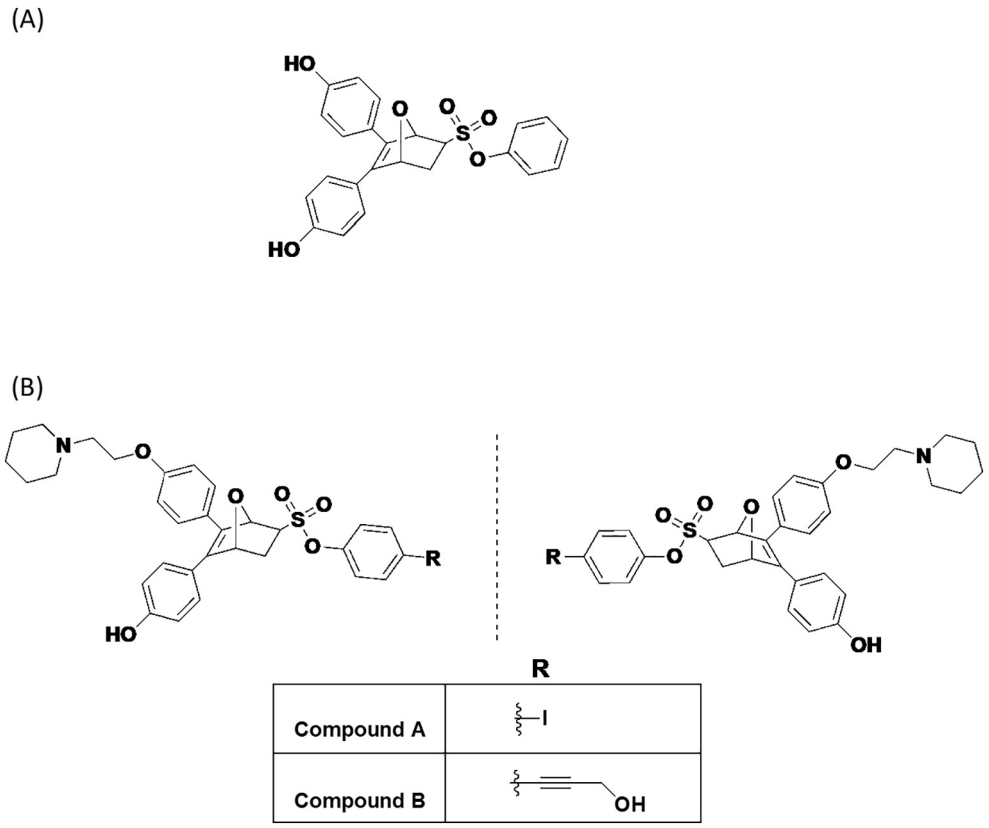

Fig. 1. OBHS ligand (A) and OBHS SERM chiral ligands (B).

chromatography and/or preparative high-pressure liquid chromatography (HPLC). The analogs were then suitably protected and selectively alkylated with 2-(*N*-piperidino) ethyl chloride at one of the 4-OH phenyl group in OBHS analogs resulting in a regiospecifically alkylated, exo diastereomer, but with a racemic mixture that needed to be further purified (Fig. 1B) [6]. The enantiomeric separation step is challenging because of the observed stereo selectivity whereby one enantiomer displays higher binding affinity than the other [7].

HPLC method development for enantiomer separation of anti-estrogens with a chiral center is a challenging task due to the presence of a highly polar and basic side chain (sometimes also referred to as SERM side chain), often containing a piperidyl, pyrrodinyl, or azetidinyl group. Sharma et al. [7] were unable to separate the OBHS enantiomers with a piperidyl side chain using various normal phase Chiralpak columns, but after shifting to Whelk-O columns having Pirkle-type chiral stationary phase, they were able to obtain the separation of the enantiomers. In our hands, OBHS analogs with additional polar groups presented issues with HPLC separation. Due to the pharmacological importance of anti-estrogens and high recent clinical interest in development of novel SERMs and SERDs, particularly for mutant forms of ER, we reasoned that a more powerful and expedient separation protocol is necessary for anti-estrogens with one or more chiral centers. OBHS derivatives provided a good test bed because of possibility of eight stereoisomers: two regioisomers when derivatized with SERM side chain, two diastereomers as *exo* and *endo* isomers, and two enantiomers.

Supercritical fluid chromatography (SFC) is widely used in pharmaceutical industries for both achiral and chiral applications [8, 9]. The unique physical and chemical properties of SF CO_2 (low viscosity, high diffusivity, low critical temperature and pressure, and good solubility in most common organic solvents) make SFC suitable for both screening and purification techniques in drug discovery, especially for molecules that are not separated easily by HPLC [10]. Here we evaluate and report enantiomeric separation of two analogs of OBHS with supercritical CO_2 modified with different modifiers and additives and using IA and IC Chiralpak stationary phases columns (CSP's). The OBHS analogs containing either an iodo group or a propargyl alcohol side chain at the para position of phenylsulfonate were synthesized using a modified scheme from a previously published procedure [6]. The *exo* and *endo* diastereomers of OBHS compounds were separated using HPLC, and then derivatized with SERM side chain by reacting 2-(*N*-piperidino) ethyl chloride with one of the 4-OH phenyl group, followed by chiral separation.

2. Experimental

2.1. Materials

ACS grade TFA, IPAm and TEA were obtained from Sigma-Aldrich (St. Louis, MO). Methanol (MeOH) and isopropanol (IPA) were HPLC grade and obtained from Thermofisher Scientific (Pittsburgh, PA). Packed columns Chiralpak IA, and IC with amylose derivatives (25 cm × 4.6 mm i.d., dp = 5 µm and 25 cm × 10 mm i.d., dp = 5 um) were purchased from Chiral Technology (West Chester, PA).

2.2. SFC/DAD and SFC/MS analysis

The SFC analysis were obtained using Thar SFC (Waters Corp., Milford, MA) system equipped with a cooled reciprocating high-pressure pump (maximum pressure of 400 atm. and maximum flow of 5 mL/min), diode array detector (DAD, 205–400 nm), auto-sampler, oven heater set to 40 °C, and a back-pressure regulator set at 120 bar. Mass spectrometry was performed using a Synapt G2 Q-Tof (Waters Corp., Milford, MA). The solvent flow was split using a pre-BPR flow Upchurch cross 1/16 PEEK splitter. Due to the high concentration of modifier used, no make-up solvent was added to the mobile phase effluent. A