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Synthesis and Discovery of Estra-1,3,5(10),6,8-pentaene-2,16 α -diol

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Abstract

A metallacycle-centered approach to the assembly of partially aromatic synthetic steroids was investigated as a means to prepare a boutique collection of unique steroidal agents. The synthesis and discovery of estra-1,3,5(10),6,8-pentaene-2,16 α -diol (**VII**) is described, along with structure–activity relationships related to its cytotoxic properties. Overall, **VII** was found to have a GI₅₀ 0.2 μ g/mL (~ 800 nM) in MDA-MB-231 human breast cancer cells, be an efficacious estrogen receptor agonist with potency for ER β > ER α (ER β EC₅₀ = 21 nM), possess selective affinity to the cdc-2-like kinase CLK4 (K_d = 350 nM), and be phenotypically related to paclitaxel by an unbiased panel assessment.

Graphical Abstract

It is widely appreciated that breakthroughs in organic chemistry have a profound impact on drug-discovery by impacting synthesis design through the establishment of new retrosynthetic relationships.¹ In short, effective means of constructing novel compositions of matter in a time- and cost-effective manner can serve as the scientific foundation to drug discovery and medicinal chemistry campaigns. With the appreciation that natural product structures continue to play what some have referred to as “a highly significant role in drug discovery and development,”² and that some classes of natural products have been a

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Supporting Information

Procedures, spectroscopic data, and details regarding all biological evaluations (growth inhibitory assays, Kinomescan, BioMap, and ER-assay information). This material is available free of charge via the Internet at <http://pubs.acs.org>.

particularly rich source of FDA-approved drugs, efforts have recently been directed toward understanding the scope and limitations associated with synthesis pathways that provide access to such compounds. In particular, compositions of matter that would well be recognized as steroidal in nature have been remarkably successful in the clinic as treatments for a wide range of indications, and this broad class of molecules is understood to be the most well-studied and successful class of natural product-inspired pharmaceuticals.³ While seeming to therefore represent a mature area of medicinal science that has thrived due to advances in organic chemistry, we believe that exploration in this area remains quite constrained due to inherent limitations associated with compound synthesis: (1) semisynthesis is effective for broad exploration of only the natural enantiomeric series,⁴ and (2) despite the myriad of approaches to *de novo* synthesis of steroids, highly efficient and flexible routes remain scarce.⁵ This perspective has fueled our curiosity in exploring this area of chemical science, and has recently led to the establishment of a new convergent strategy for the asymmetric synthesis of partially aromatic steroids.⁶ As illustrated in Figure 1, the approach features a metallacycle-mediated annulative cross-coupling reaction (**1** + **2** → **3**), regioselective cyclopropanation (**3** → **4**) and a new acid-mediated vinylcyclopropane rearrangement cascade (**4** → **5**). Appreciating that the starting enyne **2** is easily derived from epichlorohydrin, this approach has enabled asymmetric construction of steroidal species like **5** in as few as five chemical steps. Here, we describe the application of this synthetic platform as an enabling technology to fuel a search for new bioactive agents. These efforts have led to the synthesis of a boutique collection of partially aromatic steroids and the discovery of a molecule that possesses potent growth inhibitory properties in several human cancer cell lines (e.g., MDA-MB-231, AsPC-1), and has further been found to be: (1) an efficacious agonist of the estrogen receptor beta⁷ (ER β ; EC₅₀ = 21 nM) with 4-fold greater selectivity than 17- β - estradiol for ER β compared to ER α , (2) an inducer of mitotic arrest, and (3) a ligand to the cdc-2-like kinase CLK4 (K_d = 350 nM).⁸

In the course of studying our recently established synthetic pathway to partially aromatic steroids, it was observed that final acid-mediated formation of the B-ring can proceed with rearrangement.⁶ As illustrated in Figure 2A, treatment of the *p* methoxyphenyl-substituted hydrindane (**6**) with titanium tetrachloride and isopropanol in nitromethane leads to a steroidal structure whose resulting A-ring methyl ether resides at C2 rather than C3 (**7**). This observation is consistent with a mechanism for cyclization where **6** is first converted to intermediate **A** through a sequence that is thought to include protodesilylation, removal of the TBS-protecting group, generation of a cyclopropylcarbanyl cation, and selective ring opening to the furnish a homopropargylic cation (**A**).⁹ Site-selective Friedel–Crafts alkylation then is speculated to deliver spirocyclic intermediate **B**, and selective 1,2-shift of the sp² carbon rather than the dibromomethylene, rearomatization of the A-ring, and loss of HBr furnishes **7** in 54–69% yield. While mechanistically interesting, this process provides a functionalized steroidal composition that is unique in the literature and is loosely related to the natural products 17- β - estradiol and 2-methoxyestradiol (Figure 2B). Notably, 17- β - estradiol is a known agonist of the estrogen receptors α and β (ER α and ER β), and 2-methoxyestradiol has been described as a cytotoxic agent whose mechanism of action may include targeting microtubules.¹⁰

Given the growing appreciation for selective agonism of ER β as a mechanism of value for the development of anticancer agents and compounds of relevance across a diverse therapeutic landscape (i.e., cancer, neurodegeneration, schizophrenia, and neuropathic pain, among others),¹¹ and the claim that some 16-hydroxyestratrienes have selective estrogenic activity,¹² **7** was viewed as a potentially interesting intermediate of value in medicinal pursuits. In particular, this compound houses functionality that was reasoned to enable straightforward structural modification within the A, B, and D-rings (Figure 3A). In particular, the nature of **7** would allow for systematic variation at C2, C6 and C16 – molecular sites that, to our knowledge, have not been systematically explored in the context of this type of steroidal system.

The scope of potential modifications of **7** was kept small, with the goal of providing a collection of novel steroidal compositions of matter that would retain overall advantageous physical properties in the context of medicinal chemistry. For example, every compound prepared would retain at least one free alcohol to favorably impact solubility characteristics, while no modifications were considered that would greatly increase lipophilicity of any given member of the collection. The precise reactions that were embraced to transform **7** to steroidal targets of interest are depicted in Figure 3B, and include: (1) demethylation,¹³ (2) Sonogashira coupling,¹⁴ (3) Pd-catalyzed debromination, (4) lithium–halogen exchange/fluorination,¹⁵ and (5) Mistunobu reaction.¹⁶ These reaction processes were used in synthesis pathways that delivered a collection of fifteen compounds (**I** – **XV**; Figure 4) that were evaluated to identify molecules in the collection that have anticancer properties.

Without a particular biomolecular target in mind, initial assessment of each of these agents was done in simple growth inhibition assays with two human cancer cell lines: MDA-MB-231 (breast) and AsPC-1 (pancreatic). As illustrated in Figure 4, most of the compounds possessed modest GI₅₀ values in the range of ~10–40 μ M. Interestingly, compound **VII** stood out among the group, possessing a GI₅₀ value for the MDA-MB-231 human tumor cell line of ~0.2 μ g/mL (~800 nM), and a GI₅₀ value for the AsPC-1 human tumor cell line of ~0.5 μ g/mL (2 μ M). Varying stereochemistry at C16 led to a significant drop in activity (see **VIII**), while introduction of F, Br, or an alkynyl motif at C6 also negatively impacted activity (see **III**, **X**, and **XV**), as did the presence of a C2 methyl ether (see **V**).

In an effort to explore the potential medicinal value of **VII**, it was further characterized in a commercial panel of 12 human primary cell-based systems designed to model complex human tissue and disease biology of the vasculature, skin, lung and inflammatory tissues (“BioMAP Diversity PLUS panel”).¹⁷ This panel has been reasoned to offer an “unbiased means of characterizing a test agent across a broad set of systems modeling various human disease states. A test agent is used to generate a “signature profile” that can be compared to a reference database of >4,000 agents including biologics, approved drugs, chemicals and experimental agents. In this way a compound can be classified with respect to known biologically active agents as a result of an unbiased comparison across a wide variety of potential mechanisms of action. Perhaps unsurprisingly, the BioMAP analysis of compound **VII** resulted in favorable comparison to both 17- β estradiol (a dual ER α and ER β agonist) as well as 2-methoxyestradiol. However, this analysis also indicated a correlation with

several microtubule targeting agents (combretastatin A4, 2-methoxyestradiol and paclitaxel).
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With great interest in the potential value of steroid **VII** as an anticancer agent, subsequent investigation led to the discovery that it arrests cells in mitosis (see Supporting Information (SI) Fig. S16) and AutoDock simulations led to a speculation that **VII** may interact with a number of cyclin dependent kinases. As such, we moved forward to establish a profile of **VII** as a potential kinase inhibitor. Evaluation of **VII** in a panel of 468 kinases led to the conclusion that it inhibits a very small percentage of these targets ($S\text{-score}(35) = 0.03$).¹⁹ In fact, follow up K_d determinations revealed that compound **VII** has selective affinity to the cdc-2-like kinase CLK4 ($K_d = 350$ nM), with modest affinity toward CLK1, CLK2, and DYRK1A (K_d values of 1.4, 2.0, and 2.0 μM , respectively). Using an established model for inhibition of CLK1,2,4,²⁰ however, we found no evidence of CLK inhibitory activity in cells (see SI Fig. S17).

Given the structural similarity of **VII** to 17- β -estradiol, and the BioMAP analysis that grouped these agents as being phenotypically related across a broad panel of human primary cell-based systems, we assessed its effects on ER α and ER β . This effort was initiated with the knowledge that some 16-hydroxyestratrienes have been claimed to possess selective estrogenic activity, with preferential action on bone rather than the uterus.¹² While not showing activity as an antagonist at either human receptor at concentrations up to 10 μM , this unique steroidal composition proved to be a potent agonist of ER β with an EC_{50} of 21 nM (average; $n = 2$; see SI for assay details). Notably, it is also unique in that it demonstrates approximately a 4-fold shift in selectivity favoring agonism of ER β over ER α in comparison to 17- β -estradiol (Figure 5).²¹

Overall, a new approach to the *de novo* construction of partially aromatic steroids has served as an enabling technology for the discovery of a natural product-inspired agent (**VII**) that has growth inhibitory properties in several cancer cell lines, with a GI_{50} of ~ 0.2 $\mu\text{g/mL}$ (~ 800 nM) in MDA-MB-231 cells. Further analysis revealed that this agent is an efficacious agonist of the estrogen receptor with 4-fold selectivity for ER β over ER α in comparison to 17- β -estradiol, and it induces mitotic arrest in cells. An unbiased phenotypic screen (BioMAP) analysis further grouped **VII** as being related to the clinically relevant microtubule-targeting agent paclitaxel. Intriguingly, a number of publications studying ER β -agonists have reported a G2/M phase arrest,²² and perhaps these two mechanisms are related. Notably, the chemical platform that fueled this discovery also has allowed for the establishment of first-generation structure–activity relationships of **VII** that relate molecular changes at C2, C6 and C16 to growth inhibitory properties in the context of two different human tumor cancer cell lines. We are looking forward to further investigating this approach to the asymmetric synthesis of partially aromatic steroids in the context of an enabling technology for the discovery of medicinally relevant agents, as well as investigating the efficacy and mechanism of **VII** *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- (18). See Supporting Information for details concerning the KINOMEScan assay, BioMAP analysis, Kd determinations, and estrogen receptor α and β assays.
- (19). “S-score”, or “Selectivity Score”, is a quantitative measure of selectivity that is calculated by dividing the number of kinases that compounds bind to by the total number of distinct kinases tested. See Supporting Information for additional information and VII’s tree spot diagram.
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Figure 1:

Introduction.

$a = \text{available in 2-3 steps from epichlorohydrin. } b = \text{Ti}(\text{O}i\text{-Pr})_4, n\text{-BuLi. } c = \text{HCX}_3, \text{ KOH. } d = \text{TiCl}_4, \text{ MeNO}_2$

A. Vinylcyclopropane-based B-ring formation can proceed with rearrangement during the course of establishing the A-ring: Access to 2-methoxyestrans.

i = protodesilylation, deprotection, protonation of the vinylcyclopropane, and ring opening of the resulting cyclopropylcarbinyl cation. *ii* = Friedel Crafts alkylation. *iii* = selective Wagner-Meerwein rearrangement, loss of HBr and rearomatization.

B. Comparing the steroidal product **7** to known bioactive natural products: 17- β -estradiol and 2-methoxyestradiol.

Figure 2:

Acid-mediated B-ring formation with additional rearrangement, and the similarity of **7** to 17- β -estradiol and 2-methoxyestradiol.

A. Plan for exploration targeted functionality at C2, C6, and C16 stereochemistry.

B. Reactions employed for structural modification at C2, C6, and C16.

Figure 3.

Reactions employed to perturb substitution at C2, C6 and C16. NFSi = *N*-fluorobenzenesulfonimide.

Figure 4:
Novel steroids and their growth inhibitory properties [GI_{50} (μM)] in MDA-MB-231 (breast cancer) and AsPC-1 (pancreatic cancer). 40 μM was the highest concentration tested.

Figure 5.

Steroid **VII** is a selective agonist of $ER\beta$, demonstrating a 4-fold shift in comparison to 17- β -estradiol.