Synthesis and Biological Evaluations of Analogs of 2-Methoxyestradiol: New Anticancer Agents

Dissertation for the degree of Master of Pharmacy

Farhad Haidari

The Department of Pharmaceutical Chemistry
School of Pharmacy
The Faculty of Mathematics and Natural Science

UNIVERSITY OF OSLO
May 2016
Synthesis and Biological Evaluations of Analogs of 2-Methoxyestradiol: New Anticancer Agents

Dissertation for the degree of Master of Pharmacy

Farhad Haidari

The Department of Pharmaceutical Chemistry
School of Pharmacy
The Faculty of Mathematics and Natural Science

UNIVERSITY OF OSLO
2016
Synthesis and Biological Evaluations of Analogs of 2-Methoxyestradiol: New Anticancer Agents

Farhad Haidari

http://www.duo.uio.no/

Printed in Norway: Reprosentralen, Universitetet i Oslo
The lion that never had to fight

For food or water or its territory

Who stood and always got its share

Never becomes a forest king

But they lives and dies as they began

The strongest lions grow with the competitors against them

Be the best of whatever you are!

- Farhad Haidari
Acknowledgement

The task presented in this thesis was performed at the Department of Pharmaceutical Chemistry, School of Pharmacy, The Faculty of Mathematics and Natural Science, University of Oslo. The final year of pharmacy studies as a master student has presented many newly acquired experiences.

First and foremost, praises and thanks to God for being my strength. You have given me the power to believe in myself and follow my dreams. I could never have done this without the faith I have in you.

It is a genuine pleasure to express my deep sense of thanks and gratitude to my principal supervisors, Professor Trond Vidar Hansen and Associate Professor, Ander Vik. They both have been fantastic supervisors with unique knowledge of chemistry. Their dedication and keen interest above all their enormous attitudes to help their students have been solely and mainly responsible for my motivation to keep working. Their guidance, carefully monitoring and professional advice helped me largely to understand and accomplish this task.

I owe a deep sense of gratitude to all participants of the LIPCHEM research group for an enjoyable social and working environment and their support and practical advice.

I also express a deep sense of gratitude to Dr. Ove Alexander Åstrand for adequate training and instruction in the laboratory.

I am extremely thankful to my fellow students Ørjan L. Apeland, Mai M. F. El-Khatib and Daniel H. Hasselstrøm for all those conversations at the laboratory and encouragement during my work.

I take this opportunity to express my greatest regards to Koen Van Gansbeke for collaboration in the laboratory and sharing his data with me.

I would like to thank the engineers Juliana Johansen and Anne Bjerke for all help. I appreciate your support, kindness and care along the way.
Finally, and most importantly, I would like to thank my lovely wife Sumbul for her support, encouragement, quiet patience and love. My parents, Mohd Akbar and Zarguna, receive my deepest gratitude and love for their faith in me and for their co-operation, love, understanding, and constant encouragement which were the strength in carrying out the work successfully. Thanks to my sisters, Tamina and Shabana, and their family who offered invaluable support and humor over the years. Without you all, I most certainly would not be where I am today.

Oslo, May 2016

Farhad Haidari
Abstract

During the last decade, researches using 2-methoxyestradiol (2-ME) as lead compound has received great attention as a potential drug for attacking the newly formed or outgrowth of blood vessels while minimize the systemic side effects. Several 2-ME analogs have been synthesized in order to improve the biological activity and the pharmacokinetic profile by modifying the 2-, 3-, 16- and 17–position of the steroid structure. Several analogs of 2-ME have been observed to be potent inhibitor of tubulin polymerization, include ENMD-1198 (24) which was developed by Entremed. In addition, several analogs have been reported by the LIPCHEM group at University of Oslo.

In total, eight compounds were prepared using 2-methoxyestradiol, as the lead compound. An efficient approach was developed for the synthesis of the 2-ethylestradiol. These eight analogs were subjected to cytotoxic effects on the proliferation of L1210-, CEM-, HeLa- and HMEC-1, VERO and HT29 cell lines. Notably, almost all of the prepared compounds showed better activity toward CEM cell line, compared to 2-ME. Biological studies for their anti-angiogenic activity and inhibition of tubulin polymerization are currently ongoing.
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-ME</td>
<td>2-Methoxyestradiol</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>CA-1</td>
<td>Combretastatin A-1</td>
</tr>
<tr>
<td>CA-4</td>
<td>Combretastatin A-4</td>
</tr>
<tr>
<td>CAM</td>
<td>Ceric ammonium molybdate</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
</tr>
<tr>
<td>CYP450</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DBMP</td>
<td>2,6-Di-tert-butyl-4-methylpyridine</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>N, N-Dimethylformamide</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Hypoxia Inducible Factor-1 alpha</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Human umbilical vein endothelial cells</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug- resistant</td>
</tr>
<tr>
<td>MTB</td>
<td>Maximum tolerated doses</td>
</tr>
<tr>
<td>NCD</td>
<td>NanoCrystal® Dispersion</td>
</tr>
<tr>
<td>SAM</td>
<td>S-Adenosyl methionine</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
# Table of contents

Acknowledgement ........................................................................................................... V
Abstract ............................................................................................................................ VII
List of abbreviations .......................................................................................................... VIII

1 Introduction ...................................................................................................................... 1
   1.1 Cancer ....................................................................................................................... 1
   1.2 Anti-cancer agents ................................................................................................. 1
      1.2.1 Anti-angiogenetic agents .................................................................................. 2
      1.2.2 Tubulin inhibitor as anti-cancer agents .......................................................... 4
   1.3 Estrogens and 2-methoxyestradiol ......................................................................... 7
      1.3.1 Biosynthesis and the biology of 2-methoxyestradiol ........................................ 8
      1.3.2 Anti-cancer activities ....................................................................................... 9
      1.3.3 SAR-studies with 2-methoxyestradiol as lead compound .............................. 12
      1.3.4 Some results from SAR using 2-ME as lead compound ................................. 14
      1.3.5 Clinical studies .............................................................................................. 16
   1.4 Synthetic Methods ................................................................................................. 17
      1.4.1 ortho-Formylation ............................................................................................ 17
      1.4.2 Electrophilic aromatic substitution ................................................................ 18
      1.4.3 Suzuki-Miyaura coupling .............................................................................. 19
      1.4.4 Oppenauer oxidation ..................................................................................... 20
   1.5 Aim of study ............................................................................................................ 21

2 Results and discussion ................................................................................................. 22
   2.1 Method A ............................................................................................................... 24
      2.1.1 Synthesis of 2-formylestradiol (31) ................................................................. 24
      2.1.2 NMR characterization of 2-formylestradiol (31) ............................................. 24
      2.1.3 NMR characterization of 4-formylestradiol (40) ............................................. 25
      2.1.4 Synthesis of 2-vinylestradiol (32) .................................................................. 26
      2.1.5 NMR characterization of 2-vinylestradiol (32) .............................................. 26
      2.1.6 Synthesis of 2-ethylestradiol (33) .................................................................. 27
      2.1.7 NMR characterization of 2-ethylestradiol (33) .............................................. 27
   2.2 Method B ............................................................................................................... 28
      2.2.1 Reductive alkylation of estradiol (17) ............................................................. 28
2.2.2 NMR characterization of 2-ethylestradiol (33) .............................................. 28

2.3 Synthesis of 2-ethylestrone (34) ........................................................................ 29

2.3.1 NMR characterization of 2-ethylestrone (34) ................................................. 29

2.4 Synthesis of 3-tert-butylidimethylsiloxoy-2-ethylestrone (35) ....................... 30

2.4.1 NMR characterization of 3-tert-butylidimethylsiloxoy-2-ethylestrone (35) ... 30

2.5 Synthesis of (8R,9S,13S,14S)-3-((tert-butylidimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulfonate (36) ........................................................................ 31

2.5.1 NMR characterization of (8R,9S,13S,14S)-3-((tert-butylidimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulfonate (36) ........................................................................ 31

2.6 Suzuki cross-coupling ......................................................................................... 32

2.6.1 NMR characterization of 4-(((8S,9S,13S,14S)-3-((tert-butylidimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methylpyridine (37a) ......................................................................................... 33

2.6.2 NMR characterization of 4-(((8S,9S,13S,14S)-3-((tert-butylidimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-fluoropyridine (37b) ......................................................................................... 34

2.6.3 NMR characterization of 4-(((8S,9S,13S,14S)-3-((tert-butylidimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methoxypyridine (37c) ......................................................................................... 35

2.6.4 NMR characterization of 4-(((8S,9S,13S,14S)-3-((tert-butylidimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-chloropyridine (37d) ......................................................................................... 36

2.7 Deprotection of 37a-37d ....................................................................................... 37

2.7.1 NMR characterization of (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a) ......................................................................................... 37

2.7.2 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b) ................................................................. 38

2.7.3 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38c) ................................................................. 39

2.7.4 NMR characterization of (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d) ................................................................. 40

2.8 Synthesis of sulfonamides 39a-39d ........................................................................ 41

2.8.1 NMR characterization of (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39a) ......................................................................................... 41
2.8.2 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39b) .................................................................................................................. 42
2.8.3 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39c) .................................................................................................................. 43
2.8.4 NMR characterization of (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39d) .................................................................................................................. 44
2.9 Biological evaluations .......................................................................................... 45
3 Future perspectives .............................................................................................. 47
4 Conclusions ......................................................................................................... 49
5 Appendix ............................................................................................................. 50
5.1 General methods .............................................................................................. 50
5.2 Experimental procedures .................................................................................. 51
5.2.1 Method A ...................................................................................................... 51
5.2.2 Method B ...................................................................................................... 53
5.2.3 Synthesis of 2-ethylestrone (34) ................................................................... 54
5.2.4 Synthesis of 3-tert-butylidemethilsiloxy-2-ethylestrone (35) ..................... 55
5.2.5 Synthesis of (8R,9S,13S,14S)-3-((tert-butylidemethilsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulphonate (36) ................................................................. 55
5.2.6 General procedure for the Suzuki cross-coupling and synthesis of (37a-37d) .......................................................... 55 56
5.2.7 General procedure for the deprotection of (37a-37d) ............................... 59
5.2.8 General procedure for the synthesis of sulphonamides (39a-39d) ............. 62
6 References ......................................................................................................... 66
7 Appendix ............................................................................................................. 71
7.1 1H NMR and 13C NMR spectra of the synthesized compounds .................... 71
7.1.1 2-Formylestradiol (31) .................................................................................. 72
7.1.2 4-Formylestradiol (40) .................................................................................. 73
7.1.3 2-Vinylestradiol (32) .................................................................................... 74
7.1.4 2-Ethylestradiol (33) (via method A) ............................................................... 74
7.1.5 2-Ethylestradiol (33) (via method B) ............................................................... 75
7.1.6 2-Ethylestrone (34) ...................................................................................... 77
7.1.7 3-tert-Butylidemethilsiloxy-2-ethylestrone (35) .......................................... 78
7.1.8 (8R,9S,13S,14S)-3-((tert-butyldimethyl-silyloxy)-2-ethyl-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl
trifluoromethanesulfonate (36) ................................................................. 79

7.1.9 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyloxy)-2-ethyl-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methylpyridine
(37a) .............................................................................................................. 80

7.1.10 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyloxy)-2-ethyl-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-fluoropyridine
(37b) .............................................................................................................. 81

7.1.11 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyloxy)-2-ethyl-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methoxypyridine
(37c) .............................................................................................................. 82

7.1.12 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyloxy)-2-ethyl-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-chloropyridine
(37d) .............................................................................................................. 83

7.1.13 (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a) ......... 84

7.1.14 (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b) ......... 85

7.1.15 (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38c) ......... 86

7.1.16 (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d) ......... 87

7.1.17 (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39a) .... 88

7.1.18 (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39b) .... 89

7.1.19 (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39c) .... 90

7.1.20 (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39d) .... 91

7.2  HRMS of synthesized compounds ........................................................................ 92

7.2.1 (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a) ......... 92

7.2.2 (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b) ......... 93

7.2.3 (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38c) ......... 94
7.2.4 (8S, 9S, 13S, 14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-
7, 8, 9, 11, 12, 13, 14, 15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d)..................... 95
7.2.5 (8S, 9S, 13S, 14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-
7, 8, 9, 11, 12, 13, 14, 15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39a) .... 96
7.2.6 (8S, 9S, 13S, 14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-
7, 8, 9, 11, 12, 13, 14, 15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39b) .... 97
7.2.7 (8S, 9S, 13S, 14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-
7, 8, 9, 11, 12, 13, 14, 15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39c) .... 98
7.2.8 (8S, 9S, 13S, 14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-
7, 8, 9, 11, 12, 13, 14, 15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39d) .... 99
1 Introduction

1.1 Cancer

Cancer, also known as malignant neoplasms, is characterized by cells that grow out of control in an abnormally high rate.\textsuperscript{1} In the human body, cancer is a generic term for more than 100 types of diseases that can affect almost any tissue. Cancer may cause serious health consequences, and is one of the most leading causes of death worldwide,\textsuperscript{1,2} as represented by 8.2 million deaths in 2012.\textsuperscript{1}

Metastasis is a process where cancer cells invade adjoining anatomical tissues in the body and spread to other organs through the blood and the lymphatic system. The uncontrolled growth of tumor cells and their rapid proliferation makes cancer hard to heal. Therefore metastasis is the main cause of death from cancer. Lung, stomach, colorectal, esophagus and liver cancer are the most common types of cancer that kills men, while breast, lung, stomach, colorectal and cervical are the most common among women. Avoiding the main risk factors, like tobacco, could reduce cancer cases worldwide.\textsuperscript{1}

1.2 Anti-cancer agents

There have been considerable attention and progresses on cancer diagnosis and treatment. Efficient treatments have been achieved for some cancers, which have resulted in a cure or considerably prolonged the life and ensured quality of life of patients. There are many types of cancer treatment. Depending on the cancer, the most common treatments are surgery, chemotherapy, radiation therapy and biological therapy.\textsuperscript{3}

Chemotherapy is used as single agents or cytotoxic drugs in combination to destroy cancer cells and prevent spread.\textsuperscript{3,4} Achieving selectivity towards tumor cells is a challenge, because chemotherapeutic agents also act on normal rapidly multiplying cells, such as hair, bone marrow and mucous membrane cells.\textsuperscript{4}
In 1971, Dr. Folkman first proposed a concept that angiogenesis was essential for tumor invasion and metastasis (Figure 1). Inhibiting angiogenesis, the formation of blood vessels from pre-existing ones, could be essential to inhibit further tumor growth, because the supply of oxygen and nutrients support the survival of tumor cells and metastatic spread.

1.2.1 Anti-angiogenetic agents

Angiogenesis is necessary for tumor growth and spread. It is now generally accepted that to grow beyond 1–2 mm in diameter, a tumor need a dedicated blood supply. The aims of anti-angiogenic drugs in treatments are to attack the tumor by attacking the newly formed or outgrowth blood vessels and minimize the systemic side effects. There are several attacking points to inhibit angiogenesis, for example, blocking the angiogenic activators, matrix breakdown, adhesion and migration of endothelial cells.
Figure 2: Drugs with anti-angiogenic activity.

Thalidomide (1) is a compound with anti-angiogenic activity. It was first synthesized in 1953 as a sedative hypnotic drug and later in 1965 as an immunomodulatory agent.\(^8\) It was also used as a drug by pregnant women to prevent morning sickness until it was withdrawn because of reported teratogenicity from many countries.\(^10\) Thalidomide (1) and its analogs lenalidomide and pomalidomide were shown to inhibit angiogenesis and suppress tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) production. They also inhibits vascular endothelial growth factor (VEGF)– and basic fibroblast growth factor (bFGF)– induced angiogenesis.\(^8,10\)

Bevacizumab (Avastin®) (2), a recombinant humanized monoclonal IgG antibody by DNA technology, was the first U.S. Food and Drug Administration (FDA)-approved angiogenesis inhibitor in the treatment of colorectal cancer. Since it first approval in February 2004, bevacizumab (2) has been approved for lung cancer in 2006, for metastatic breast cancer in 2008 and for renal and glioblastoma cancers in 2009.\(^11\) Bevacizumab (2) binds to VEGF and thereby prevents VEGF-interaction with VEGF-receptors. The inhibition of VEGF signaling may affect tumor growth and progression through several mechanisms, including endothelium cell survival, migration and proliferation and increased vascular permeability.\(^12\)

The specific indication, metastatic breast cancer, was withdrawn by the FDA on November, 2011 after concluding that Avastin® (2) has not been shown to be safe and effective for treating breast cancer. The regulations required additional clinical trials that confirm that there
is a significant clinical benefit to patients such as increased survival. Unfortunately, Avastin® (2) failed to verify a clinical benefit in the required confirmatory studies.\textsuperscript{11}

1.2.2 Tubulin inhibitor as anti-cancer agents

Natural products are the main source for anti-cancer drugs. They are privileged structures with inherent biological activities. A survey done by Newman and Cragg has shown that natural products have long been used in treatment of cancer.\textsuperscript{13} About 60\% of all anti-cancer drugs developed before year 2002 are formally derived from a natural product. Among those, 20\% are synthetic with a pharmacophore derived from a natural product.\textsuperscript{14}

\textbf{Figure 3: Examples of natural anti-cancer agents with anti-mitotic activities.}
Microtubules are among the most well-known targets for anti-cancer therapy. They are long, tube-shaped composed of polymerized tubulin proteins, α- and β-tubulin heterodimers. Microtubules are important components of the cytoskeleton and are essential for several cellular functions including cell shape, intracellular transport, cell division and etc.\textsuperscript{15}

Interaction with the tubulin is one of the most important mechanisms of action of natural products.\textsuperscript{16} Anti-mitotic agents interacts with tubulin through at least four binding sites: the laulimalide, taxol, vinca alkaloid, and colchicine sites (\textit{Figure 4}).\textsuperscript{17}

\textbf{Figure 4: Tubulin binding site of microtubule targeting agents (MTA)}\textsuperscript{17}

At the beginning of the 1960s, paclitaxel (Taxol\textsuperscript{TM}, 3) became the first interesting compound of the taxane family, when a crude extract of the bark from the Pacific yew tree, \textit{Taxus brevifolia}, showed significant anti-tumor activity.\textsuperscript{14} Later, the interaction of Taxol\textsuperscript{TM} (3) within a specific site on β-subunit of tubulin was reported by Dr. Susan Horwitz. Taxol\textsuperscript{TM} (3) showed extremely good activity in animal testing in the early 1970s and entered Phase I clinical trials in 1984. In 1992, the U.S. FDA approved Taxol\textsuperscript{TM} (3) for the treatment for ovarian cancer, and for use against refractory or anthracycline-resistant breast cancer in 1994.\textsuperscript{18} In addition to causing the stabilization of the polymerized microtubule, Taxol\textsuperscript{TM} (3) inhibits the transition from the G\textsubscript{0} to the S-phase in human fibroblast. Taxol\textsuperscript{TM} has also shown to inhibit many functions of human neutrophils, decrease the production of TNF-α and the proliferation of vascular endothelial cells.\textsuperscript{14}

Other natural products known to bind to the taxol site are epothilones (4 and 5), discodermolide (6) and eleutherobin (7). Laulimalide (8), a cytotoxic natural product isolated from several species of marine sponges, shows potent antiproliferative activities against both
multidrug-resistant (MDR) and non-MDR human cancer cell lines. Laulimalide (8) binds to a different site than the taxol, but induce tubulin polymerization and stabilization.\textsuperscript{19}

The vinca alkaloids belong to one of the most important classes of anti-cancer agents. Vinca alkaloids were isolated from \textit{Catharanthus roseus} (L.) G. Don belonging to the family \textit{Apocynaceae}. Since their discovery in the 1950s, the two natural drugs vinblastine (9) and vincristine (10) have been widely clinically used in cancer chemotherapy. The leaf extracts was first tested by R. Noble, which had a little effect on blood glucose levels, but shared a strong effect on bone marrow and white blood cell counts. Later, reproducible anti-tumor activity was observed by J. Svoboda and co-workers at Lilly Laboratories. Anti-angiogenic activities of the vinca alkaloids were discovered in the early 1990s.\textsuperscript{20} Vinca alkaloids bind to tubulin in the vinca domain with high affinity and inhibit its polymerized form, microtubules, which play important roles in mitosis and meiosis through formation of the spindle that separates the chromosomes.\textsuperscript{14,20} Vinblastine (9) and vincristine (10) at close to their maximum tolerated doses (MTB) have great tumor vascular effects.\textsuperscript{9}

Rhizoxin (11) is a potent anti-mitotic agent against human and murine tumor cells. It was isolated from pathogenic plant fungus, \textit{Rhizopus chinensis} in 1984. It has been evaluated in clinical trials, but has not been developed into an anti-cancer drug.\textsuperscript{16}

Several compounds bind to the colchicine site, including colchicine (12), podophyllotoxin (13), combretastatin A-4 (14), and 2-methoxyestradiol (16). Colchicine (12) was the first destabilizing agent extracted from \textit{Colchicum autumnale} \textit{L}. Colchicine (12) is an effective inhibitor of mitosis, but because of its low therapeutic index and toxicity it is not used as anti-cancer agent.\textsuperscript{17}

Podophyllotoxin (13) was first isolated in 1880 from rhizome of \textit{Podophyllum},\textsuperscript{21,22} and is an aryltetralinlactone cyclolignan with a flat, rigid five-ring system.\textsuperscript{22} Podophyllotoxin (13) was reported to be a mitosis-inhibiting agent, by inhibit formation of mitotic spindle, binding the microtubules and induce mitotic arrest in metaphase. It binds strongly to tubulin, at the same site as colchicine (12), but the binding is more rapidly and reversible. Podophyllotoxin failed to be used as anti-cancer agent because of undesirable toxic side-effects, but modifications of its structure led to the less toxic anti-cancer agents, etoposide and teniposide.\textsuperscript{22}
The Combretaceae plant family includes about 600 species of trees and shrubs in 20 genera. The *Combretum* genus with 250 species includes the largest number, of which some 24 species are well-known in African folk medicine. Several combretastatins have been isolated from the African willow tree *Combretum caffrum*. Combretastatin A-4 (CA-4, 14) was the most potent cytotoxic natural product in this family that binds to the colchicine site on tubulin causing inhibition formation of microtubules in cells. The promising results for CA-4 (14) have allowed for more pharmacophore studies, which have been beneficial for development of combretastatins A-1 series. The more soluble sodium-phosphate salt of CA-4 (14) and CA-1 (15) has been developed, that converts rapidly by endogenous phosphatases to CA-4 (14) and CA-1 (15). The CA-1 (15) prodrug has shown to be more potent than the CA-4 (14) prodrug in preclinical models.

1.3 Estrogens and 2-methoxyestradiol

Estrogens were identified as “the woman’s hormone” produced and secreted mainly by the ovaries and partly by the adrenals in the female body. Androgens are common precursors of estrogens. In 1941, estrogen products were approved by the U.S. FDA as hormone replacement therapy for postmenopausal symptoms. Estrogens plays key role in female reproductive system, cardiovascular, skeletal, and central nervous systems. In the mid of 1990s, about 38% of postmenopausal women in the United States used estrogen products to treat symptoms of menopause and prevent chronic conditions such as heart disease, osteoporosis and Alzheimer’s disease.

The metabolism of 17β-estradiol results in the formation of several metabolites that retain various degrees of estrogenic activity. 2-Methoxyestradiol (2-ME, 16), see Figure 3, is an endogenous metabolite of 17β-estradiol having 500-3200 fold lower affinity for estrogen receptors than 17β-estradiol, respectively. The anti-angiogenetic activities of 2-ME are not shared by 17β-estradiol, which approves that such actions are estrogen receptor-independent. On the other hand, the daily oral administration of 2-ME to rats and dogs for 28 days showed estrogenic activity. This estrogenic activity possibly due to metabolites of 2-ME and not 2-ME itself. The steroid 2-ME is a potent inhibitor of tumor vasculature and cell growth. 2-ME is under investigation in clinical trials because of its potent anti-angiogenic activity. Biological activities of 2-ME such as anti-cancer and anti-angiogenetic effects will be discussed in Section 1.3.2.
1.3.1 Biosynthesis and the biology of 2-methoxyestradiol

The estrogen 2-ME is a naturally occurring metabolite, produced by hydroxylation by cytochrome P450 enzymes, and consecutively, o-methylation by catechol-O-methyltransferase (COMT), see Scheme 1. The cytochrome P450-dependent conversion of 17β-estradiol and estrone (18) at the 2-position is catalyzed by phase I enzymes CYP1A1/2 and CYP3A, which is the major metabolic pathway in the liver. The same biochemical process involves also hydroxylation at C4, which is catalyzed by CYP3A, yielding the regioisomeric 4-methoxyestradiol compound (not depicted). The 2-hydroxyl metabolites 19 and 20 are further metabolized by a COMT, by transferring a methyl group from the naturally occurring compound, S-Adenosyl methionine (SAM), to the 2-OH group and converts these products to 2-ME and 2-methoxyestrone (21), see Scheme 1. The catechol-estrogen metabolite 21 is subsequently reduced at the 17-position, producing 2-ME. The phase II metabolism of 2-ME is mainly by glucuronidation and sulfation, yielding the conjugates 22 and 23, respectively. The semiquinone radical intermediate (not depicted), formed by a metabolic oxidation-reduction step, may damage the DNA or the other cellular compounds if these reactive metabolites are not deactivated by glutathione conjugation. The 4-OH estradiol has been linked with kidney cancer in Syrian male hamsters.

COMT is a ubiquitous enzyme crucial to catechol metabolism occurring in many tissues in the body including liver, kidney, brain, lung, etc. Since COMT is present in different organs, 2-ME can be produced in many tissues. The exact plasma concentrations of 2-ME are not known, but are estimated to be in the picomolar range under physiological conditions. During late pregnancy the concentration of 2-ME can increase by 1000-fold.
1.3.2 Anti-cancer activities

The steroid 2-ME was considered to be an inactive metabolite of 17β-estradiol (17), but during the last few decades, 2-ME has received attention due to its anti-cancer activities and is a new promising anti-cancer agent that has entered clinical trial.\textsuperscript{32} It exhibits potent apoptotic activity against rapidly growing tumor cells. The anti-angiogenic activity of 2-ME through a direct apoptotic effect on both endothelial and tumor cells have been demonstrated \textit{in vitro} and \textit{in vivo}.\textsuperscript{26,33}

These vascular-targeting therapies fall into two general categories: the angiogenesis inhibitors (AIs) and the vascular-disrupting agents (VDAs). AIs are compound that inhibits the tumor-initiated angiogenic process. VDAs are compounds that affects development of new blood vessels or damage the established tumor vasculature.\textsuperscript{34} The compound 2-ME displays both anti-angiogenic and vascular-disrupting properties. The exact \textit{in vivo} mechanism of action of 2-ME is still unclear, but it has been shown to inhibit the growth of various tumor cells. Several mechanisms of action have been reported based on pharmacological studies, including microtubule stabilization by inhibition of the colchicine site that will be discussed below.\textsuperscript{26,33} An overview of the cell lines inhibited by 2-ME, with the inhibition concentration values reported is shown in \textit{Table 1}.\textsuperscript{26}
Table 1: Cell lines inhibited by 2-methoxyestradiol.\textsuperscript{26}

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IC\textsubscript{50}-values* (µmol/L)</th>
<th>Cell Type</th>
<th>IC\textsubscript{50}-values* (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung (HOP-62)</td>
<td>0.7</td>
<td>HUVEC</td>
<td>0.45</td>
</tr>
<tr>
<td>Lung (H460)</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung (A549)</td>
<td>5.0</td>
<td>Lung (Lewis lung, murine)</td>
<td>1.68</td>
</tr>
<tr>
<td>Colon (HCT-116)</td>
<td>0.47</td>
<td>Melanoma (B16BL6, murine)</td>
<td>0.4</td>
</tr>
<tr>
<td>CNS (SH-SY5Y)</td>
<td>1.3</td>
<td>Melanoma (B16F10, murine)</td>
<td>0.3</td>
</tr>
<tr>
<td>CNS (SF-539)</td>
<td>0.32</td>
<td>Endothelial (EOMA, murine)</td>
<td>0.89</td>
</tr>
<tr>
<td>Melanoma (UACC-62)</td>
<td>0.36</td>
<td>Endothelial (H5V, murine)</td>
<td>1.0</td>
</tr>
<tr>
<td>Ovarian (OVCAR-3)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal (SN12-C)</td>
<td>0.95</td>
<td>Lung (V79, hamster)</td>
<td>3</td>
</tr>
<tr>
<td>Prostate (DU-145)</td>
<td>1.8</td>
<td>Ovarian (granulose, porcine)</td>
<td>3</td>
</tr>
<tr>
<td>Breast (MDA-MB-435)</td>
<td>0.08–0.61</td>
<td>Smooth muscle (aorta, rabbit)</td>
<td>1</td>
</tr>
<tr>
<td>Breast (MDA 231)</td>
<td>1.03</td>
<td>Adipocytes (murine)</td>
<td>1.7</td>
</tr>
<tr>
<td>Breast (MCF-7)</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoblast (Jurkat)</td>
<td>0.3</td>
<td>Brain capillary (bovine)</td>
<td>0.19–0.49</td>
</tr>
<tr>
<td>Lymphoblast (TK6)</td>
<td>1–2</td>
<td>Pulmonary artery (bovine)</td>
<td>0.5</td>
</tr>
<tr>
<td>Lymphoblast (WTK1)</td>
<td>1–2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human nontumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin fibroblast (HFK2)</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*IC\textsubscript{50}-value: Half maximal inhibitory concentration. The inhibitor concentration required to cause 50% enzyme inhibition in the presence of a substrate.

**Anti-tubulin and anti-proliferative activity**

The anti-proliferative effects of 2-ME were first reported in the 1980s.\textsuperscript{28} The anti-tumor and anti-growth mechanisms involve several cell specific pathways including disruption of microtubules, cytokines and growth arrest.\textsuperscript{35} The steroid 2-ME has shown to affect cell division and act directly on microtubules by interacting with colchicine-binding site of β-tubulin.\textsuperscript{36} It has been reported that 2-ME is a competitive inhibitor of colchicine-binding to tubulin with a K\textsubscript{i} value of 22 ± 2 µM.\textsuperscript{36} The compound 2-ME exhibits anti-proliferative effects a threshold concentration of 0.1–0.3 µM, with maximum inhibitory effects between 10
and 20 µM in most cell types, including endothelial and tumor cells.\textsuperscript{28} In most cell types, 2-ME inhibits cell cycle arrest at G\textsubscript{1}, as a result of DNA synthesis inhibition. Inhibition of tubulin polymerization leads to cell cycle arrest at G\textsubscript{2}/M cell cycle checkpoint, possibly progressing to apoptosis.\textsuperscript{28,37,38} The steroid 2-ME induces apoptosis in actively proliferating cells,\textsuperscript{28,39} such as tumor and activated endothelial cells, but does not induce apoptosis in normal epithelial cells. The compound has shown to induce p53-independent apoptosis in leukemia cells leading to oxidative-stress.\textsuperscript{28} The steroid 2-ME is a potent inhibitor of endothelial cell proliferation and migration, with a concentration up to 3-5 µM.\textsuperscript{40}

**Anti-angiogenetic activity**

The steroid 2-ME is the first steroid to have high anti-angiogenic activity by itself. The anti-angiogenetic activity of 2-ME was discovered in the mid of 1990s. The compound 2-ME showed potent inhibition on different types of endothelial cells \textit{in vitro}, the half-maximal inhibitory concentration (IC\textsubscript{50}) being \textasciitilde0.15 µM.\textsuperscript{40} Oral administration of 2-ME in mice showed to suppress the growth of tumor and inhibit the formation of functional microvascular networks.\textsuperscript{28} Expression of VEGF, which is an important cytokine of angiogenesis with critical roles in tumor angiogenesis and is inducible by hypoxia, is a multistage process in which hypoxia inducible factor-1 alpha (HIF-1\textalpha) plays a key role. HIF-1\textalpha is a transcription factor controlling angiogenesis, which is overexpressed in more than 70% of human cancers and their metastases, as compared to their adjacent normal tissue.\textsuperscript{41}

The steroid 2-ME inhibits angiogenesis directly through anti-proliferative and apoptotic effects. The compound has also indirect effects through inhibition of HIF-1\textalpha expression, consequently inhibiting VEGF expression in endothelial and tumor cells.\textsuperscript{28,41} This was shown in prostate and breast cancers cells.\textsuperscript{41} Inhibition of growth factor production, VEGF, is likely to lead to a reduction in tumor neovascularization.\textsuperscript{28} Inhibition by 2-ME of HIF-1\textalpha requires higher concentrations compared with concentrations needed to inhibit cell proliferation and induce apoptosis.\textsuperscript{42} Some of the suggested mechanisms involve the activation of caspases\textsuperscript{26,38}, induced production of superoxides\textsuperscript{26}, and increased expression of B-cell lymphoma 2 (Bcl-2) and Fas ligand in endothelial cells.\textsuperscript{28}
The biological activities of 2-ME have led to considerable research using 2-ME as lead compound. The steroid 2-ME metabolizes quickly to the relatively inactive metabolite 2-methoxyestrone (21), explaining the short half-life \textit{in vivo} and poor bioavailability. The very short half-life in rodents (less than 30 minutes) has been explained by its phase II metabolism. Several 2-ME analogs have been synthesized in order to improve the biological activity and the pharmacokinetic profile by modifying the 2-, 3-, 16- and 17–position of the steroid structure.

When analogs containing various substituents in the 2-position were investigated, it was observed those containing unbranched chain substituents containing either carbon, nitrogen or oxygen atoms from the 2\textsuperscript{nd} row of the periodic table gave maximum anti-tubulin activity. The two most potent analogs were 2-ethoxyestradiol with an IC\textsubscript{50}-value of 0.90 µM and 2-((E)-1-propenyl)-estradiol with IC\textsubscript{50}-value of 1.1 µM compared with 2-ME with IC\textsubscript{50}-values ranging from 3.0 to 17 µM. The cytotoxic activity drops as the chain is either lengthened or shortened, but it may also reflect the large size of the atom.
In order to improve the bioavailability of 2-ME due to rapid phase I and phase II metabolism, modifications of the C-3 position were examined.\textsuperscript{44,47} A hydrogen donor in the 3-position seems to be critical for the interactions with tubulin, but there are also other important factors such as $\pi$-electrons as part of hydrogen acceptor groups and the size of the substituent.\textsuperscript{47} Suwandi et al.\textsuperscript{47} reported that the replacement of the 3-OH group of 2-ME by hydrogen donor substituents, such as 3-NHCN and 3-NHCONH$_2$, provided analogs with good anti-proliferative activity against both human umbilical vein endothelial cells (HUVEC), and human breast carcinoma MDA-MB-231 cells.

As the C-17 position is exposed to metabolism through oxidation and conjugation,\textsuperscript{48-50} several 2-ME analogs with polar, alkyl, endocyclic and exocyclic olefins have been done on the area of the molecule.\textsuperscript{49,50} Three modified analogs of 2-ME, 17-methylene, 17-ethylene and 16,17-endocyclic olefin exhibits better anti-proliferative activity compared to 2-ME. As for the C-17 position, the increase in length of the carbon chain showed significantly lower activity since bulky groups are not well tolerated.\textsuperscript{49} It has also been observed that as steric bulk increases in C-16 position, the IC$_{50}$-value for MDA-MB-231 cell line proliferation also increases. The 16-methyl and ethyl analogs showed same activity as 2-ME, but there was a 35-45 fold drop in activity with larger substituents. Contrary, the larger 16-substituents showed to be more potent in HUVEC cell line.\textsuperscript{45}

\textbf{Figure 6:} An overview of SAR-studies with 2-methoxyestradiol as a lead compound.
The activity of 2-ME towards tubulin polymerization is based on the competitive binding studies with colchicine (12). Other types of modifications to increase the interaction of 2-ME with colchicine site are based on the structural similarities between colchicine (12) and 2-ME. The A-ring of 2-ME is suggested to correspond to the C-ring of colchicine (12) and the A-ring of colchicine is functionally equivalent to C- and D-rings of 2-ME.

### 1.3.4 Some results from SAR using 2-ME as lead compound

Several analogs of 2-ME were modified at the C-3 and C-17 positions to improve metabolic stability and anti-tubulin properties. One best example that has emerged from these efforts, contained a 16, 17 olefin and an amide group at C-3 position, is ENMD-1198 (24). The lead analog, ENMD-1198 (24), showed increased plasma concentration, and was 2.5-6-fold more potent than 2-ME at inhibiting human endothelial cell proliferation. ENMD-1198 (24) was developed by Entremed and has entered clinical trials.

Our research group LIPCHEM at the School of Pharmacy, University of Oslo, has prepared several new analogs of 2-ME and some analogs were observed to be potent inhibitor of tubulin polymerization and angiogenesis, see Table 2 and Figure 7 for structures.

**Table 2: Biological evaluation of 2-ME and analogs 25-30.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>K562 cell assay IC₅₀ (µM)ᵃ</th>
<th>Anti-angiogenesis IC₅₀ (µM)ᵇ</th>
<th>Tubulin polymerization (%)ᵇ</th>
<th>Tubulin polymerization Inhibition IC₅₀ (µM)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.9</td>
<td>0.6</td>
<td>4.1ᶜ</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0.4</td>
<td>0.7</td>
<td>2.2ᶜ</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0.7</td>
<td>0.6</td>
<td>2.1ᶜ</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.4</td>
<td>109</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>0.2</td>
<td>41</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.1</td>
<td>51</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>2-ME</td>
<td>0.8</td>
<td>3.2</td>
<td>2.2–3.5</td>
<td></td>
</tr>
<tr>
<td>Colchicine (12)</td>
<td>n.d.</td>
<td>100</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Results of three experiments performed as triplicates.
ᵇ Determined at 10 µM.
ᶜ Results of three experiments performed as duplicates. n.d. = not determined
The 4-substituted pyridine analog 25 and the 6-substituted isoquinoline analogs 26-27 were as active as, or slightly more active, than 2-ME in the leukemia cell line K562. The position of the nitrogen atom is shown to be important for the activity of 2-ME analogs. All three compounds 25-27 were more potent as inhibitors against anti-angiogenesis compared to 2-ME, with IC₅₀-values 0.6, 0.7 and 0.6 µM, respectively. The most potent tubulin polymerization inhibitors were 26 (IC₅₀ = 2.2 µM) and 27 (IC₅₀ = 2.1 µM). The compound 28 showed inhibition of tubulin polymerization better than colchicine (12), 109% vs 100%. The three most potent compounds 28-30 exhibit anti-angiogenetic activity with IC₅₀-values below 0.5 µM. The analogs with both a 2-ethyl- and 3-sulfonamide substituent on the A-ring has shown to be the common structural properties for the most potent analogs. Of interest, no significant estrogen activity was observed.

\[\text{Figure 7: Structure of 2-ME analogs.}\]
1.3.5 Clinical studies

ENMD-1198 (24) has reached phase I and II clinical trials in patients with solid tumors. The clinical studies of ENMD-1198 (24) showed both anti-angiogenic and vascular disruptive properties. In addition, ENMD-1198 (24) was shown to having the ability to protect the bone against breast cancer-induced osteolysis.56

The promising preclinical results of 2-ME have led to further investigation in clinical trials28,48 for the treatment of breast and prostate cancer.28 The clinical trials show that 2-ME is generally well tolerated, and oral doses up to 1.2 g daily showed no toxicity effects.26,28 Results from the clinical study showed a clinically significant reduction in bone pain in some patients. The patients in the 1000 mg dose group experienced hot flashes.26

A phase II multicenter, double-blind, randomized, placebo-controlled study of two dosages of 2-ME capsules (400 and 1200 mg/day) taken place at Indiana University and the University of Wisconsin Comprehensive Cancer Center. Thirty-three men with hormone-refractory prostate cancer were involved into this phase II study. In totally four patients, grades 2 and 3 liver transaminase elevation was observed. 2-ME has limited oral bioavailability. However, it was concluded that 2-ME was well tolerated. Moreover, a decrease in prostate-specific antigen was observed.57

Low aqueous solubility is the major problem encountered with formulation and often require high dose in order to reach therapeutic plasma concentration after oral administration. Poor water solubility and significant hepatic first pass metabolism is possible explanation for poor bioavailability of 2-ME. A new formulation known as 2-ME NanoCrystal® Dispersion (NCD) was reported to improve its limited bioavailability.44,58 Based on the preclinical studies of 2-ME NCD, a phase I study in patients with advanced solid malignancies was conducted.58 The treatment was generally well tolerated at the oral dose of 1000 mg every 6 hours.44,58 In addition, safety and efficacy of 2-ME NCD was assessed in a phase II clinical trial in 18 patients with recurrent, platinum-resistant or refractory ovarian cancer. The 2-ME NCD formulation was well tolerated and showed better bioavailability.59
1.4 Synthetic Methods

1.4.1 ortho-Formylation

Several methods for formylation of aromatic reactions are available, but most of them involve the use of noxious reagents and harsh reaction conditions. In 1999, Skattebøl and Hofsløkken published a simplified version of the Casiraghi ortho-formylation. It has similarities with an electrophile aromatic substitution of hydrogen in an aromatic compound. Their ortho-formylation of phenol uses the reagents magnesium chloride, triethylamine and paraformaldehyde, and provides high yields of salicylaldehydes. The reaction mechanism for the Skattebøl-Hofsløkken ortho-formylation is assumed to be similar to that suggested by Casiraghi et al., see Scheme 2.

![Scheme 2](image)

*Scheme 2: The reaction pathway for ortho-formylation of phenols using the Skattebøl-Hofsløkken method.*
1.4.2 Electrophilic aromatic substitution

In 2015, an organic reaction in which an electrophilic aromatic substitution of aldehyde, in the presence of a thiol promoter, which can either be reduced or reacted further, was developed by Parnes and Pappo. It is a simple and highly chemoselective and regioselective method for introducing alkyl substituents into phenols and electron rich aromatic systems. This reaction enables the direct introduction of both aromatic and a variety of aliphatic groups, including linear and branched groups, into arenes.

![Scheme 3: Reductive alkylation reactions and its application on 17β-estradiol (17).](image-url)

---

**Scheme 3:** Reductive alkylation reactions and its application on 17β-estradiol (17).
1.4.3 Suzuki-Miyaura coupling

The Suzuki-Miyaura coupling reaction is an organic reaction where organoboron compounds reacts with organotriflates or organohalides in the presence of a palladium complex to form carbon-carbon bonds, see Scheme 4.\textsuperscript{64} The availability of the reagents and the mild conditions offers several additional advantages, such as tolerating a wide range of functional groups and being performed in the presence of water. Furthermore, the inorganic by-products are non-toxic and easily removed from the reaction mixture.\textsuperscript{65}

\textit{Scheme 4: The mechanism of the Suzuki-Miyaura coupling reaction.}\textsuperscript{64}
1.4.4 Oppenauer oxidation

The Oppenauer oxidation is a reaction named after the Austrian chemist Rupert Viktor Oppenauer. This reaction is used for selectively oxidizing secondary alcohols to ketones, using acetone or cyclohexanone as the hydride acceptor in presence of aluminum alkoxide, see Scheme 5. In the first step, the alcohol is bound to the aluminum, making a complex and then gets deprotonated. Then in the next step, both the substrate alcohol and cyclohexanone are bound to the aluminum. The aluminum activates cyclohexanone for the hydride transfer from the alkoxide and the desired ketone is formed.

Scheme 5: Mechanism of the Oppenauer Oxidation.
1.5 Aim of study

The aim of this study was to synthesis new analogs of 2-ME based on previous SAR-studies of this promising anticancer agent. It is known that the 2-, 3-, 16- and 17-position on the aromatic A- and D-ring are significant for bioavailability and anti-cancer activities. By making a new series of analogs with modifications both at the 17-position and at the A-ring, more information on the SAR of 2-ME will became available.

The methoxy group in the 2-position was replaced with an ethyl substituent and different substituted pyridine rings were introduced at the 17-position. All analogs were prepared both with a hydroxy- and a sulfonamide group at the 3-position, see Figure 8. The analogs were prepared from commercially available 17β-estradiol (17), with key synthetic steps described in Section 1.4.

Those compounds are currently under biological testing at National Research Institute of Chinese Medicine, Taipei, Taiwan and in Leuven, Belgium, by professor Sandra Liekens.

![Figure 8: General structure of analogs with modifications both at the A- and D-ring.](image-url)
2 Results and discussion

The synthetic strategies for the synthesis of the 2-ethyl substituted analogs are shown in Scheme 6. Compound 33 from 17β-estradiol (17) were prepared using the ortho-formylation of phenols, as described in Section 1.4.1, yielding the desired 2-formylestradiol 18. The Skattebøl ortho-formylation RX was invest by Akselsen and Hansen. Then a Wittig-reaction between 18 and the ylide of Wittig reagent, afforded the alkene 32. The last step was a reduction of the double bond in 32 in the presence of palladium on carbon and hydrogen gas, giving 2-ethylestradiol 33.

Later, a new method was found to introduce the 2-ethylsubstituent on the estradiol moiety in the presence of thiol promoter in 2,2,2-trifluoretanol, providing compound 33 in a much shorter way.

Further modifications of the 17-position was achieved in a three-step protocol, yielding compounds 37a-37d. An Oppenauer oxidation in the presence of a mixture of aluminum isopropoxide and cyclohexanone in toluene followed by TBS-protection of the phenol in 34, provided the ketone 35. Then compound 35 was converted to the enol triflate 36.

A Suzuki-Miyaura coupling reaction between the triflate 36 and organoboron in the presence of a palladium complex, gave compounds 37a-37d. The deprotection of the TBS-group yielded the analogs 38a-38d. Finally, the introduction of the sulfonamide in the 3-position was achieved, to afford products 39a-39d.
Scheme 6: Synthesis of the 2-ethyl substituted estrogen analogs 38a-38d and 39a-39d.
2.1 Method A

This method is a three-step synthesis of 2-estrolestradiol (33) from 17β-estradiol (17) in good yields, as previously reported by Solum et al.\textsuperscript{55} The key steps are an ortho-formylation reaction followed by a Wittig reaction and then a reduction of the resulting alkene.

2.1.1 Synthesis of 2-formylestradiol (31)

The first step in the synthesis was an ortho-formylation reaction with 17β-estradiol (17) as substrate in the presence of three equivalents of magnesium chloride, three equivalents triethylamine and four equivalents of para-formaldehyde in refluxing THF. Two regioisomeric products 31 and 40 were obtained in favor of the desired estrogen 31. The pure product 31 was obtained in 75% yield after chromatography. The ratio has previously been reported by Akselsen and Hansen to be 13:1 (31:40).\textsuperscript{68} The pure product 40 is isolated and reported for the first time as a white solid in 2% yield.

![Synthesis of 2-formylestradiol (31)]

2.1.2 NMR characterization of 2-formylestradiol (31)

In the $^1$H NMR, the most downfield signal at 10.78 ppm, integrating for one proton, comes from the phenol due to hydrogen bonding with aldehyde in C2-position. The next deshielded peak at 9.81 ppm (1H) arises from the proton of the aldehyde. The two separated singlets at 7.43 ppm (1H) and 6.70 ppm (1H) appears from the two aromatic protons. The signal for the proton in the C17 can be seen as a triplet (1H) at 3.75 ppm. The singlet at 0.80 ppm integrating for 3H is a characteristic signal for the C18 methyl group. A multiplet at 2.83-2.96 ppm can be seen which integrates for 2H. This is assumed to be from the hydroxy-group in C17, which overlaps with the signal of one of the aliphatic protons. The remaining protons can be seen as multiplet signals between 1-3 ppm.
The $^{13}$C NMR spectra show 19 carbon signals, which are in agreement with the compounds formula (C$_{19}$H$_{24}$O$_3$). The peak at 196.5 ppm is in the range of a carbonyl carbon, which is representative of aldehyde carbon at C2. The peaks between 100-160 ppm are in the range of aromatic carbons, and the peak at 159.7 ppm is suggestive of C3 since the hydroxy group shifts the peak further downfield. The peak at 82.2 ppm refers to C17, since C17 is next to an alcohol functional group. The remaining 11 aliphatic carbons can be seen between 20-55 ppm, which is corresponding with the compounds total carbons.

The obtained $^1$H NMR and $^{13}$C NMR spectra are in agreement with the earlier published data.$^{55}$

### 2.1.3 NMR characterization of 4-formylestradiol (40)

The most downfield signal at 11.98 ppm, integrating for one proton, comes from the phenol due to hydrogen bonding with aldehyde in C2-position. The next deshielded peak at 10.38 ppm, (1H) arises from the proton of the aldehyde. The two doublet peaks at 7.49 ppm ($J = 8.9$ Hz, 1H) and 6.79 ppm ($J = 8.8$ Hz, 1H) appears from the two ortho aromatic protons. The size of the coupling constant is typical for an ortho-coupling on an aryl. The signal for the proton in the C17 can be seen as a triplet (1H) at 3.74 ppm. The singlet at 0.79 ppm integrating for 3H is a characteristic signal for C18 methyl group. A multiplet occurred at 3.28-3.36 ppm integrates for 1H is most likely the hydroxy-group in C17. The remaining protons can be seen as multiplet signals between 1-3 ppm.

The $^{13}$C NMR spectra show 19 carbon signals, which are in agreement with the compounds formula (C$_{19}$H$_{24}$O$_3$). The peak at 196.0 ppm is representative of aldehyde carbon at C4. The peaks between 100-160 ppm are in the range of aromatic carbons, and the peak at 161.9 ppm is suggestive of C3 due to the deshielding effect of the hydroxy group. The peak at 82.2 ppm is from C17. The peak at 11.5 ppm is methyl carbon at C18, since primary alkanes comes most upfield. The remaining 10 aliphatic carbons can be seen between 20-55 ppm, which is corresponding with the compounds total carbons.

The obtained $^1$H NMR and $^{13}$C NMR spectra are in compliance with the earlier published data.$^{55}$
2.1.4 Synthesis of 2-vinylestradiol (32)

The second step was a Wittig reaction between 2-formylestradiol (17) and the mixture of methyltriphenylphosphonium bromide and potassium tert-butoxide in THF. This afforded the alkene 32. Purification yielded the product as a white solid in 79% yield.

2.1.5 NMR characterization of 2-vinylestradiol (32)

The two separated singlets at 7.29 ppm (1H) and 6.52 ppm (1H) shows the two aromatic protons. The most significant peaks at 6.89 ppm, 5.68 ppm and 5.29 ppm, represents the three olefinic protons in the vinyl group. The peak at 6.89 ppm (dd, \( J = 17.8 \) and 11.2 Hz, 1H) is from the proton coupling with geminal proton and the vicinal trans protons. The two doublet of doublet peaks at 5.68 ppm (\( J = 17.7 \), 1.4 Hz, 1H) and 5.29 ppm (\( J = 11.2 \), 1.4 Hz, 1H) are indicative for the geminal protons. The triplet signal at 3.74 ppm integrating for one proton accounts for the proton in the C17. Another significant peak is at 0.79 ppm, representing the protons on the C18 methyl group, since the integration value is also about 3. The multiplet at 2.77-2.83 ppm (2H) is believed to be from the hydroxy-group in C17, overlapping with the signal of one of the aliphatic protons. The remaining protons can be seen as multiplet signals between 1-3 ppm.

The \(^{13}\)C NMR spectra show as expected 20 carbon signals. The peaks between 100-160 ppm are in the range of aromatic and alkene carbons, which are characteristic for aromatic ring and olefinic carbons. The peak at 151.2 ppm is from C3 since it is directly bonded with a hydroxy group. The peak at 82.4 ppm refers to C17, since an alcohol functional group shifts the peak further downfield. The peak at 11.5 ppm is methyl carbon at C18. The remaining 10 aliphatic carbons can be seen between 20 – 55 ppm, which is corresponding with the remaining carbons.
2.1.6 Synthesis of 2-ethylestradiol (33)

The last step was a reduction of the double bond in 2-vinylestradiol (32) using hydrogen gas in the presence of palladium on carbon in ethyl acetate. The reaction mixture was stirred in room temperature overnight. That gave the desired 2-ethylestradiol (33) in 71% yield after purification.

2.1.7 NMR characterization of 2-ethylestradiol (33)

The most downfield signals at 7.06 ppm and 6.50 ppm, integrating for one proton each, arise from the two protons on the aromatic ring. The phenol group is shown at 4.63 ppm as a singlet (1H). The signal for the proton in the C17, which is directly bonded to the oxygen, can be seen as a triplet (1H) at 3.75 ppm. The ethyl substituent gives a quartet (2H) at 2.60 ppm and a triplet (3H) at 1.23 ppm. The multiplet at 2.77-2.87 ppm (2H) is suggested to be from the hydroxy-group in C17, overlapping with the signal of one of the aliphatic protons. The remaining protons can be seen as multiplet signals between 1-3 ppm.

The $^{13}$C NMR spectra show 20 carbon signals, which is in agreement with the number of carbon atoms in this compound. As expected, the six aromatic carbons resonate between 100-160 ppm. The C17 carbon is most likely the signal at 82.4 ppm, because oxygen exhibits deshielding effect on the carbon. The most upfield signal at 11.5 ppm comes from C18 methyl carbon. The remaining 12 aliphatic carbons can be seen between 20-55 ppm.

The obtained $^1$H NMR and $^{13}$C NMR spectra are in good agreement with the earlier published data for this compound.\textsuperscript{55}
2.2 Method B

2.2.1 Reductive alkylation of estradiol (17)

A simple and highly chemo- and regioselective method was used to introduce an ethyl-group on estradiol (17). The method is based on an electrophilic aromatic substitution, reported very recently by Parnes and Pappo.\(^6\) 17β-estradiol (17) was reacted at 50 °C with 2.5 mol % copper(II) triflate, three equivalents of acetaldehyde and six equivalents of ethanethiol in 2,2,2-trifluoretnanol for 16 hours. Then three equivalents of triethylsilane was added and the mixture was stirred for 3-5 hours at 50 °C. After evaporation the residual product 33 was purified by flash chromatography and obtained in a 41-71% yield as white solid. This method is an efficient method or shorter route that saves a lot of time.

2.2.2 NMR characterization of 2-ethylestradiol (33)

The \(^1\)H and \(^{13}\)C spectra look identical with the recorded data, see section 2.1.7. The only difference is that the signal for phenolic group is not detected here. CDCl\(_3\) is slightly acidic (CHCl\(_3\) has a pKa of about 15.7), and in such solutions, acidic hydroxy-groups can exchange these protons with the deuterium from the deuterated solvent. This may explain why the \(^1\)H signal of the phenol is not observed.

The obtained \(^1\)H NMR and \(^{13}\)C NMR spectra are in good agreement with the earlier published data for this compound.\(^5\)
2.3 Synthesis of 2-ethylestrone (34)

2-Ethylestradiol (33) was oxidized to 2-ethylestrone (34) using an Oppenauer oxidation. The 2-ethylestradiol (33) was dissolved in toluene in a round bottomed flask equipped with a Dean-Stark condenser, added five equivalents of aluminum-isopropoxide and forty equivalents of cyclohexanone and heated at reflux overnight. Excess cyclohexanone was removed by distillation, and the residue was purified by column chromatography to give the product 34 in 55% yield.

2.3.1 NMR characterization of 2-ethylestrone (34)

The two aromatic protons give two signals at 7.03 ppm (1H) and 6.52 ppm (1H). The signal for the C17 proton can be seen as a triplet (1H) at 3.74 ppm. The peaks at 2.60 (q, J = 7.5 Hz, 2H) and 1.21 (t, J = 7.5 Hz, 1H) is most likely from the ethyl substituent in the C2. The C18 methyl group can be seem as singlet at 0.90 ppm integrating for 3H. The remaining protons from the compound appear in the aliphatic region between 1-3 ppm.

In the $^{13}$C NMR spectra, the six aromatic carbons resonate between 100-160 ppm. The most downfield peak at 222.0 ppm accounts for the ketone carbon at C17. The peak at 14.8 and 23.4 ppm seems to be from the ethyl substituent at C2. The peak at 14.2 ppm is from the methyl carbon in the C18. The 10 aliphatic carbons resonate between 20-55 ppm.

In addition the $^1$H spectra show signals integrating for a total of 20 protons. These are the multiplet at 3.6 ppm, integrating for two protons, a multiplet at 1.96 ppm which integrates for four protons and a multiplet at 1.52 ppm integrating for 14 protons. The recorded $^{13}$C NMR shows four extra signals, at 70.8, 35.8, 25.8 and 24.5 ppm. These signals correspond with the signals from cyclohexanol, and are thus probably caused by trace amounts of this substance. Cyclohexanol is formed during the Oppenauer oxidation reaction.
The obtained $^1$H NMR and $^{13}$C NMR spectra are in compliance with the earlier published data.\textsuperscript{55}

## 2.4 Synthesis of 3-\textit{tert}-butyldimethylsilox-2-ethylestrone (35)

The phenol in 2-ethylestrone (34) was protected with a TBS-group to give 3-\textit{tert}-butyldimethylsilox-2-ethylestrone (35) using \textit{tert}-butyldimethylsilyl chloride in the presence of imidazole in dry dimethylformamide. The reaction mixture was stirred at room temperature for four hours before aqueous acidic work-up. Flash chromatography afforded the TBS-protected 2-ethylestrone as a colorless oil in 54\% yield.

\[
\begin{array}{c}
\text{HO} \quad \text{Ketone} \\
\text{34} \quad \text{TBS-Cl}
\end{array}
\]

\[
\begin{array}{c}
\text{Imidazole, DMF} \\
\text{54\%}
\end{array}
\]

\[
\begin{array}{c}
\text{35}
\end{array}
\]

### 2.4.1 NMR characterization of 3-\textit{tert}-butyldimethylsilox-2-ethylestrone (35)

The $^1$H NMR of 35 is very similar to that previously reported, with the exception of additional signals from the \textit{tert}-butyldimethylsilyl group. This group displays a singlet (9H) at 1.01 ppm and a singlet (6H) at 0.23 ppm. The two aromatic protons give signal at 7.06 ppm (1H) and 6.50 ppm (1H), from the \textit{tert}-butyl group and the two methyl groups bound to silicone, respectively. The peaks at 2.57 (q, $J = 7.6$ Hz, 2H) and 1.17 (t, $J = 7.5$ Hz, 1H) is probably from the ethyl substituent in the C2. The C18 methyl group can be seen as singlet at 0.91 ppm (3H). The last protons from the compound appear in the aliphatic region between 1-3 ppm.

In the $^{13}$C spectra, the ketone carbon at C17 resonates at 221.4 ppm. The six aromatic carbons can be seen between 100-160 ppm, and the peak at 151.8 ppm is suggestive of C3 due to the deshielding effect. The peak at 15.1 ppm accounts for the terminal carbon in the ethyl substituent. The peak at 14.3 ppm is from methyl carbon in the C18. The strong signal at 26.2 ppm must be from the \textit{tert}-butyl group. The signal from the two diastereotopic methyl groups
attached to silicon atom is visible as two signals at -3.6 ppm. The remaining 11 aliphatic carbons resonate between 15-55 ppm.

The obtained $^1$H NMR and $^{13}$C NMR spectra are in compliance with the earlier published data.$^{55}$

### 2.5 Synthesis of (8R,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulfonate (36)

The transformation of TBS-protected 2-ethylestrone (35) into the enol triflate (36) was performed using $N$-phenyl-bis (trifluromethanesulfonimide) and potassium-bis(trimethylsilyl)amide in dry THF. The mixture was stirred at -78 °C for three hours, before it was purified by column chromatography yielding the pure product as a colorless oil in 64% yield.

![Synthesis of (8R,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulfonate (36)](image)

### 2.5.1 NMR characterization of (8R,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulfonate (36)

In the $^1$H NMR spectra the two aromatic protons give singlets at 7.04 ppm (1H) and 6.51 ppm (1H). The signal for the olefinic proton at C16 can be seen as a doublet of doublet ($J = 3.3, 1.7$ Hz, 1H) at 5.63 ppm. The peaks from the ethyl group at C2 gives a quartet at 2.59 ($J = 7.5$ Hz, 2H), and a triplet at 1.19 ppm ($J = 7.5$ Hz, 3H). The C18 methyl group can be seen as singlet at 1.03 ppm integrating for 3H. The two singlets at 1.01 ppm (9H) and 0.23 ppm (6H)
arise from the TBS-protecting group. The last 13 protons from the compound appear in the aliphatic region between 1-3 ppm, which is in agreement with the total of 39 protons in the compound.

In the recorded $^{13}$C NMR spectra there are eight peaks between 100-160 ppm, which are characteristic for the six aromatic and two olefinic carbon atoms. The most intense peak at 26.3 ppm accounts for the three equivalent carbons in the tert-butyl group. The CF$_3$ carbon signal should be split into a quartet of intensities 1:3:3:1 due to the effect of the three fluorine nuclei. The $^{19}$F-$^{13}$C give low intensity signals because of the quartet splitting and are not affected by enjoy the large interaction involved in the direct magnetic coupling between nuclei as CH groups. The two peaks at 120.6 and 117.5 ppm is suggested to be the two strongest signals from the quartet, and there are also two minor peaks that could be the two weakest signals from the quartet. The peaks at 15.1 ppm and 15.8 ppm are possibly from the terminal carbon in the ethyl substituent and methyl carbon in the C18. The two peaks at -3.6 ppm and -3.7 ppm comes from the two diastereotopic methyl groups attached to silicon atom. The remaining 11 aliphatic carbons resonate between 15-55 ppm.

In addition the $^1$H and $^{13}$C spectra show extra signals. In the $^1$H NMR spectra, there is a singlet peak at 2.18 ppm and in the recorded $^{13}$C NMR, there are two extra signals, at 207.1 and 31.3 ppm. These signals correspond with the signals from acetone.\textsuperscript{71}

The obtained $^1$H NMR and $^{13}$C NMR spectra are in compliance with the earlier published data.\textsuperscript{55}

### 2.6 Suzuki cross-coupling

The TBS protected steroid triflate 36 was reacted in a Suzuki–Miyaura cross-coupling reaction with the numbered commercially available boronic acids (Scheme 6), affording the four desired products 37a–37d as light yellow oils in 53–86% yields. The triflate 36 was reacted with two equivalents cesium carbonate and the boronic acid (1.05 equiv.) using tetrakis(triphenylphosphine)palladium(0) in a 1:1 mixture of water and THF. The reaction mixture was stirred overnight at 60 °C under argon.
2.6.1 NMR characterization of 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methylpyridine (37a)

The most downfield peak at 8.41 ppm (1H) is a doublet with \( J = 5.2 \) Hz, this proton has only one proton ortho to it. It must be for the proton in the C6-position in the pyridine ring. The singlet at 7.16 ppm (1H) is most likely for the proton in the C3 in the pyridine ring. The doublet of doublet at 7.11 ppm (\( J = 5.3, 1.6 \) Hz, 1H) is formed by coupling of one proton to both an ortho and a meta proton, which must be the proton from the C5 in the pyridine ring. The two singlets at 7.05 ppm (1H) and 6.51 ppm (1H) resonate for the two protons in the aromatic A-ring. The signal for the olefinic proton at C16 can be seen as a multiplet at 6.18-6.16 ppm (1H). A multiplet at 2.62-2.55 ppm can be seen which integrates for 5H. This is thought to be from the CH\(_2\)-group in the ethyl substituent, which overlaps with the signal of the methyl-group in pyridine ring. The peak from the three terminal protons in the ethyl group at C2 gives a triplet at 1.18 ppm (\( J = 7.5 \) Hz, 3H). The C18 methyl group can be seen as singlet at 1.08 ppm integrating for 3H. The singlet at 1.02 ppm (9H) and the doublet at 0.24 ppm (6H) arise from the TBS protecting group. The last 13 protons from the compound appear in the aliphatic region between 1-3 ppm.
The $^{13}$C NMR spectra show 13 peaks between 100-160 ppm, which are in agreement with the total of 11 aromatic and two olefinic carbon atoms. The most intense peak at 26.2 ppm accounts for the three equivalent carbons in the tert-butyl group. The peaks at 18.7 ppm, 17.2 ppm and 15.1 ppm are possibly from the ethyl substituent and methyl carbon in the C18. The two peaks at -3.6 ppm and -3.6 ppm comes from the two diastereotopic methyl groups attached to silicon atom. The remaining 11 aliphatic carbons resonate between 20-60 ppm, which are consistent with the total of 29 carbons in the compound.

2.6.2 NMR characterization of 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-fluoropyridine (37b)

In the recorded $^1$H NMR spectra the peak at 8.13 ppm (d, $J = 5.3$ Hz, 1H) must be for the proton in the C6-position in the pyridine ring. The multiplet at 7.20-7.17 ppm (1H) and the singlet at 7.06 ppm (1H) are probably for the proton in the C3 and C5 in the pyridine ring. The two peaks at 6.92 ppm (s, 1H) and 6.52 ppm (s, 1H) arises for the two protons in aromatic ring A. The peak for the olefinic proton at C16 can be seen as a multiplet at 6.28-6.25 ppm. The peaks at 2.60 (q, $J = 7.4$ Hz, 2H) and 1.20 (t, $J = 7.5$ Hz, 1H) is probably from the ethyl substituent in the C2. The C18 methyl group can be seem as singlet at 1.09 ppm (3H). The singlet at 1.03 ppm (9H) and the doublet at 0.25 ppm (6H) come from the TBS protecting group. The last 13 protons from the compound appear in the aliphatic region between 1-3 ppm.

The $^{13}$C NMR spectra show 19 peaks between 100-170 ppm. The peaks at 165.8 and 163.5 ppm are suggestive of C2 in the pyridine ring. The peaks at 152.4 ppm (d) and 150.5 ppm (d) are possibly from C4 and C6 in the pyridine ring. The doublets at 106.7 ppm and 119.4 ppm
are most likely from C3 and C5 in the pyridine ring. The six carbons in the aromatic ring A and olefinic carbons can be seen between 100-160 ppm, and the peak at 151.7 ppm is suggestive of C3 due to the deshielding effect. The most intense peak at 26.2 ppm accounts for the three equivalent carbons in the tert-butyl group. The peaks at 18.6 ppm, 17.2 ppm and 15.1 ppm are possibly from the ethyl substituent and methyl carbon in the C18. The two peaks at -3.6 ppm and -3.7 ppm comes from the two diastereotopic methyl groups attached to silicon atom. The remaining 10 aliphatic carbons resonate between 20-60 ppm.

2.6.3 NMR characterization of 4-((8S,9S,13S,14S)-3-((tert-butyl(dimethyl)silyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methoxypyridine (37c)

In the $^1$H NMR spectra the most downfield peak at 8.08 ppm (1H) is a doublet with $J = 5.4$ Hz. This peak is for the proton in the C6-position in the pyridine ring. The signal at 7.05 ppm (s, 1H) is most likely for the proton in the C3 in the pyridine ring. The doublet of doublet at 6.91 ppm ($J = 5.5$, 1.5 Hz, 1H) is the proton from the C5 in the pyridine ring. The two aromatic protons in the A-ring give the two singlets at 6.76 ppm (1H) and 6.50 ppm (1H). The C16 olefinic proton gives a multiplet at 6.17-6.16 ppm (1H). The singlet at 3.96 ppm (3H) is most likely from the methoxy group. The ethyl group at C2 gives a quartet at 2.58 ppm and a triplet at 1.18 ppm. The C18 methyl group is detectable as singlet at 1.06 ppm. The singlet at 1.02 ppm integrating for 9H and the doublet at 0.24 ppm integrating for six hydrogens appears from the TBS protecting group. The last 13 protons from the compound can be seen as multiplet signals in the aliphatic region between 1-3 ppm.
In the recorded $^{13}$C NMR spectra 13 peaks are shown between 100-160 ppm, which are in agreement with the total of 11 aromatic and two olefinic carbons. The signal at 57.2 ppm is probably from the methoxy group. The most intense peak at 26.2 ppm accounts for the three equivalent carbons in the *tert*-butyl group. The peaks at 18.7 ppm, 17.2 ppm and 15.1 ppm are most likely from the ethyl substituent at C2 and C18 methyl carbon. The two signals most upfield at -3.6 ppm and -3.6 ppm arises from the two methyl groups attached to silicon. The last 11 carbons resonate between 20-60 ppm, which are in agreement with the compound formula (C$_{32}$H$_{45}$NO$_2$Si).

**2.6.4 NMR characterization of 4-(((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-chloropyridine (37d)**

In the recorded $^1$H NMR spectra the peak at 8.30 ppm (d, $J = 5.2$ Hz, 1H) is from the proton in the C6-position in the pyridine ring. The signal at 7.33 ppm (s, 1H) is probable for the proton in the C3, and the peak at 6.91 ppm (dd, $J = 5.3$, 1.5 Hz, 1H) is the proton from the C5 in the pyridine ring. The two aromatic protons give two singlets at 7.05 ppm and 6.51 ppm. The C16 olefinic proton gives a multiplet at 6.26-6.23 ppm (1H). The ethyl group at C2 gives a quartet at 2.59 ppm and a triplet at 1.18 ppm. The C18 methyl group is visible as singlet at 1.07 ppm. The singlet at 1.02 ppm (9H) and the doublet at 0.24 ppm (6H) arise from the TBS protecting group. The remaining protons can be seen as multiplet signals in the aliphatic region between 1-3 ppm.

The signals from the carbons in the pyridine ring and the aromatic A-ring and the two olefinic carbons arise between 100-160 ppm. The peak at 26.2 ppm comes from the three carbons in
the tert-butyl group. The peaks at 18.7 ppm, 17.2 ppm and 15.1 ppm are presumably from the ethyl substituent at C2 and methyl carbon at C18. The two most upfield signals at -3.6 ppm and -3.6 ppm arises from the two methyl groups attached to silicon in TBS group. The last carbons resonate between 20-60 ppm, which are in agreement with total of 31 carbons in the compound.

2.7 Deprotection of 37a-37d

The deprotection of the TBS protected steroids 37a-37d was performed using tert-butylammoniumfluoride in THF. The reaction mixture was stirred at room temperature under argon atmosphere overnight. This gave the desired products 38a-38d in 45-88% yield after purification.

2.7.1 NMR characterization of (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a)

The doublet at 8.41 ppm (1H) is from the proton in the C6-position in the pyridine ring. The singlet at 7.17 ppm (1H) is probably for the proton in the C3 in the pyridine ring. The signal at 7.12 ppm (dd, J = 5.3, 1.6 Hz, 1H) is from the proton from the C5 in the pyridine ring. The
two protons on the A-ring are visible at 7.06 ppm (1H) and 6.55 ppm (1H). The phenol group is shown at 6.30 ppm as a singlet (1H). The signal for olefinic proton at C16 can be seen as a multiplet at 6.18-6.16 ppm (1H). The ethyl substituent at C2 gives a quartet at 2.64 ppm and a triplet at 1.24 ppm. The methyl group in the pyridine ring gives a singlet at 2.57 ppm (3H). The C18 methyl group is visible as a singlet at 1.08 ppm. The remaining protons can be seen as multiplet signals in the aliphatic region between 1-3 ppm.

The $^{13}$C NMR spectra show 13 peaks between 100-160 ppm, which are in agreement with the total of aromatic and olefinic carbons. The peak at 24.7 ppm is suggested to be from the methyl carbon in the C2 in the pyridine ring. The peaks at 23.6 ppm, 17.2 ppm and 14.9 ppm are most likely from the ethyl substituent and methyl carbon in the C18. The remaining 10 aliphatic carbons resonate between 20-60 ppm, which are consistent with the total of 26 carbons in the compound.

2.7.2 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b)

In the $^1$H NMR spectrum the most downfield doublet at 8.13 ppm (1H) arises from the proton in the C6-position in the pyridine ring. The multiplet at 7.18 ppm (1H) is most likely from the proton in the C5 and the singlet at 7.05 ppm from the proton from the C3 in the pyridine ring. The aromatic ring A protons are detectable at 6.92 ppm (1H) and 6.54 ppm (1H). The C16 proton resonates as a multiplet at 6.28-6.24 ppm. The signal for phenol group arises at 4.79 ppm as a singlet (1H). The quartet at 2.62 ppm and the triplet at 1.24 ppm arise from the ethyl group in the C2. The peak for C18 methyl group comes as a singlet at 1.08 ppm. The last protons can be seen in the aliphatic region between 1-3 ppm.
In the recorded $^{13}$C NMR spectra 19 peaks are shown between 100-170 ppm. The peaks at 165.8 and 163.5 ppm are suggestive of C2 in the pyridine ring. The peaks at 152.3 ppm (d) and 150.6 ppm (d) are possibly from C4 and C6 in the pyridine ring. The peaks at 106.7 ppm (d) and 119.4 ppm (d) are most likely from C3 and C5 in the pyridine ring. The six carbons in the aromatic ring A and two olefinic carbons can be seen between 100-160 ppm, and the peak at 151.8 ppm is most likely C3 in the ring A. The peaks at 23.5 ppm, 17.2 ppm and 14.8 ppm are possibly from the ethyl substituent and methyl carbon in the C18. The remaining nine aliphatic carbons resonate between 20-60 ppm.

2.7.3 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38c)

The C6 proton in the pyridine ring arises as doublet at 8.08 ppm. The singlet at 7.05 ppm (1H) is probably for the proton in the C3 in the pyridine ring. The C5 proton in the pyridine ring gives a doublet of doublet signal at 7.12 ppm ($J = 5.3, 1.6$ Hz, 1H). The two aromatic ring protons arise at 6.76 ppm and 6.52 ppm. The C16 olefinic proton gives a multiplet at 6.20-6.14 ppm (1H). The phenol group can be seen at 4.85 ppm as a singlet (1H). The methoxy group gives a singlet at 3.96 ppm (3H). The ethyl substituent at C2 gives a quartet at 2.62 ppm ($J = 7.5$ Hz, 2H) and a triplet at 1.24 ppm ($J = 7.5$ Hz, 3H). The C18 methyl group arises as a singlet at 1.08 ppm (3H). The remaining 13 protons can be seen as multiplet signals between 1-3 ppm.

The 13 peaks between 100-160 ppm show the total of aromatic and olefinic carbons. The signal at 57.2 ppm is most likely from the methoxy group. The peaks at 23.5 ppm, 17.2 ppm
and 14.9 ppm are suggested to be from the ethyl substituent and methyl carbon in the C18. The remaining 10 aliphatic carbons resonate between 20-60 ppm, which are consistent with the total of 26 carbons in the compound.

2.7.4 NMR characterization of (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d)

The product 38d was not stable to silica gel. This compound was tried to purify by flash chromatography two times, but still collecting fractions gave no sign of it. A number of things could have happened here. 1) The compound decomposed on the column and never came off. 2) The compound came off in the solvent front. 3) The compound came off, but the fractions are so dilute that it hasn’t been detected. It couldn’t be the 2) and 3) point, because all the fractions were evaporated in vacuo after flash chromatography. The third time the residue was checked by TLC and ¹H NMR and was taken further to next step without purifying.

In the recorded ¹H NMR spectrum the peak at 8.29 ppm (d, J = 5.2 Hz, 1H) is from the proton in the C6-position in the pyridine ring. The signal at 7.35-7.30 ppm (m, 1H) is probable for the proton in the C3 and the peak at 7.22 ppm (dd, J = 5.2, 1.5 Hz, 1H) is the proton from the C5 in the pyridine ring. The two aromatic protons give two singlets at 7.04 ppm and 6.54 ppm. The C16 olefinic proton gives a multiplet at 6.29-6.22 ppm (1H). The phenolic group can be seen at 4.82 ppm as a singlet (1H). The ethyl group at C2 gives a quartet at 2.62 ppm (J = 7.5 Hz, 2H) and a triplet at 1.25 ppm (J = 7.5 Hz, 3H). The singlet at 1.07 ppm (3H) arises from the C18 methyl group. The remaining 13 protons can be seen as multiplet signals in the aliphatic region between 1-3 ppm.
The introduction of the sulfonamide group on the estrogens 38a-38d was achieved using 2,6-di-tert-butyl-4-methylpyridine (DBMP) and sulfamoyl chloride in dry dichloromethane. The mixture was stirred at room temperature overnight, before the residues were purified by flash chromatography yielding the pure product as colorless solids in 42-90% yield.

### 2.8.1 NMR characterization of (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39a)

In the $^1$H NMR spectrum the most downfield doublet at 8.49 ppm (1H) is from the proton in the C6-position in the pyridine ring. The multiplet at 7.18 ppm (2H) is probably for the proton in the C3 in the pyridine ring and the C1 in the A ring. The signal at 7.14 ppm (m, 1H) is from the proton from the C5 in the pyridine ring. The proton in the C4 on the A-ring is visible at 7.12 ppm (1H). The signal for olefinic proton at C16 can be seen as a triplet ($J = 2.5$ Hz, 1H) at 6.22 ppm. The protons from the sulfonamide group are shown at 5.16 ppm as a singlet (2H). The ethyl substituent at C2 gives a quartet ($J = 7.5$ Hz, 2H) at 2.71 ppm and a triplet ($J = 7.6$ Hz, 3H) at 1.24 ppm. The methyl group in the pyridine ring gives a singlet at 2.59 ppm.
The C18 methyl group gives a singlet at 1.08 ppm (3H). The remaining protons can be seen as multiplet signals in the aliphatic region between 1-3 ppm.

In the recorded 13C NMR spectra 24 signals are shown instead of 26 due to low amount of product in the NMR sample. There are 11 signals for aromatic and olefinic carbons showing between 110-160 ppm. The peak at 23.9 ppm is suggested to be from the methyl carbon in the C2 in the pyridine ring. The peaks at 23.0 ppm, 16.7 ppm and 14.6 ppm are most likely from the ethyl substituent and methyl carbon in the C18. The remaining nine aliphatic carbons resonate between 20-60 ppm, which are consistent with the total of 26 carbons in the compound.

2.8.2 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39b)

The doublet at 8.12 ppm (1H) arises from the proton in the C6-position in the pyridine ring. The multiplet at 7.17 ppm (2H) is probably from the proton in the C5 and the C3 in the pyridine ring. The aromatic ring A protons are detectable at 7.11 ppm (1H) and 6.91 ppm (1H). The C16 proton resonates as a multiplet at 6.31-6.23 ppm (1H). The sulfonamide group protons are visible at 5.05 ppm as a singlet (2H). The quartet at 2.71 ppm and the triplet at 1.23 ppm arise from the ethyl group in the C2. The peak for C18 methyl group comes as a singlet at 1.08 ppm. The last protons can be seen in the aliphatic region between 1-3 ppm.

In the recorded 13C NMR spectra 19 peaks are shown between 100-170 ppm. The peaks at 165.4 ppm and 163.4 ppm are suggestive of C2 in the pyridine ring. The peaks at 152.2 ppm (d) and 150.5 ppm (d) are possibly from C4 and C6 in the pyridine ring. The peaks at 106.7
ppm (d) and 119.4 ppm (d) are most likely from C3 and C5 in the pyridine ring. The six carbons in the aromatic ring A and two olefinic carbons can be seen between 100-160 ppm. The peaks at 23.5 ppm, 17.1 ppm and 15.0 ppm are possibly from the ethyl substituent and methyl carbon in the C18. The remaining nine aliphatic carbons resonate between 20-60 ppm.

2.8.3 NMR characterization of (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39c)

In the recorded $^1$H NMR spectrum the C6 proton in the pyridine ring arises as doublet at 8.09 ppm with $J = 5.4$ Hz. The sulfonamide group protons are observable at 7.93 ppm as a singlet (2H). The singlet at 7.19 ppm (1H) is probably for the proton in the C3 in the pyridine ring. The C5 proton in the pyridine ring gives a doublet of doublet signal at 7.02 ppm ($J = 5.4$, 1.5 Hz, 1H). The two aromatic ring protons arise at 7.03 ppm (1H) and 6.75 ppm (1H). The C16 olefinic proton gives a multiplet at 6.34-6.29 ppm (1H). The methoxy group gives a singlet at 3.85 ppm (3H). The triplet at 1.13 ppm ($J = 7.5$ Hz, 3H) is the signal from the three terminal protons in the ethyl substituent. The C18 methyl group arises as a singlet at 1.02 ppm (3H). The remaining 15 protons can be seen as multiplet signals between 1-3 ppm.

In the $^{13}$C NMR spectrum 13 peaks between 100-170 ppm show the total of aromatic and olefinic carbons. The peaks at 163.9 ppm and 151.7 are suggestive of C2 in the pyridine ring and C3 in the aromatic ring A. The peaks at 23.5 ppm, 17.1 ppm and 15.0 ppm are possibly from the ethyl substituent and methyl carbon in the C18. The remaining nine aliphatic carbons resonate between 20-50 ppm, which are consistent with the total of 26 carbons in the compound.
2.8.4 NMR characterization of (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39d)

In the recorded $^1$H NMR spectra the peak at 8.31 ppm (d, $J = 5.2$ Hz, 1H) is from the proton in the C6-position in the pyridine ring. The signal at 7.51 ppm (s, 1H) is probable for the proton in the C3 and the peak at 7.46 ppm (dd, $J = 5.3$, 1.5 Hz, 1H) is the proton from the C5 in the pyridine ring. The two aromatic protons give two singlets at 7.25 ppm and 7.14 ppm. The C16 olefinic proton gives a multiplet at 6.47-6.44 ppm (1H). The ethyl group at C2 gives a quartet at 2.76 ppm and a triplet at 1.25 ppm. The singlet at 1.16 ppm (3H) arises from the C18 methyl group. The remaining protons can be seen as multiplet signals in the aliphatic region between 1-3 ppm.

The $^{13}$C NMR spectra show 13 peaks between 100-160 ppm, which are in agreement with the total of aromatic and olefinic carbons. The peaks at 29.9 ppm, 28.6 ppm and 27.5 ppm are most likely from the ethyl substituent and methyl carbon in the C18. The remaining eight aliphatic carbons resonate between 20-60 ppm, which are consistent with the total of 24 carbons in the compound.
2.9 Biological evaluations

The final compounds 38a-38d and 39a-39d were evaluated for their cytotoxic effects on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM), human cervix carcinoma cells (HeLa), human dermal microvascular endothelial cells (HMEC-1), African green monkey kidney epithelial cells (VERO) and human colon adenocarcinoma cells (HT29), see Table 3. The compounds that exhibited the lowest IC$_{50}$ values in all six cancer cell lines were the compounds 39a-39d, which all contain a sulfonamide group in the 3-position. The most potent compound of these was compound 39d, which carry a 2-chloropyridine-4-yl group in the 17-position. This compound displayed good activity towards all six cancer cell lines. Among the analogs containing a phenol in the 2-position, the most active were compounds 38a and 38c. These two compounds were especially active towards the CEM cell line.

Table 3: Biological evaluations of analogs 38a-38d and 39a-39d.

<table>
<thead>
<tr>
<th>Compound</th>
<th>L1210 IC$_{50}$ (µM)*</th>
<th>CEM IC$_{50}$ (µM)*</th>
<th>HeLa IC$_{50}$ (µM)*</th>
<th>HMEC-1 IC$_{50}$ (µM)*</th>
<th>VERO IC$_{50}$ (µM)*</th>
<th>HT29 IC$_{50}$ (µM)*</th>
<th>cLogP$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>38a</td>
<td>65 ± 1</td>
<td>56 ± 6</td>
<td>32 ± 4</td>
<td>&gt; 100</td>
<td>38 ± 6</td>
<td>55 ± 13</td>
<td>6.80</td>
</tr>
<tr>
<td>38b</td>
<td>≥ 100</td>
<td>78 ± 17</td>
<td>≥ 100</td>
<td>&gt; 100</td>
<td>28 ± 7</td>
<td>36 ± 11</td>
<td>6.53</td>
</tr>
<tr>
<td>38c</td>
<td>42 ± 3</td>
<td>35 ± 4</td>
<td>39 ± 5</td>
<td>62 ± 1</td>
<td>≥ 100</td>
<td>≥ 100</td>
<td>7.12</td>
</tr>
<tr>
<td>38d</td>
<td>≥ 100</td>
<td>90 ± 7</td>
<td>≥ 100</td>
<td>&gt; 100</td>
<td>≥ 100</td>
<td>≥ 100</td>
<td>7.10</td>
</tr>
<tr>
<td>39a</td>
<td>2.6 ± 0.7</td>
<td>1.7 ± 0.0</td>
<td>4.1 ± 2.6</td>
<td>9.0 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>6.09</td>
</tr>
<tr>
<td>39b</td>
<td>2.2 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.9 ± 0.0</td>
<td>4.5 ± 2.7</td>
<td>2.0 ± 0.3</td>
<td>8.4 ± 0.6</td>
<td>5.82</td>
</tr>
<tr>
<td>39c</td>
<td>4.0 ± 0.4</td>
<td>2.0 ± 0.4</td>
<td>6.7 ± 0.2</td>
<td>10 ± 1</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>6.41</td>
</tr>
<tr>
<td>39d</td>
<td>1.1 ± 0.1</td>
<td>0.40 ± 0.07</td>
<td>1.6 ± 0.2</td>
<td>2.3 ± 0.5</td>
<td>0.39 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>6.39</td>
</tr>
<tr>
<td>MT-53</td>
<td>9.6 ± 5.5</td>
<td>2.0 ± 0.0</td>
<td>72 ± 16</td>
<td>64 ± 41</td>
<td></td>
<td></td>
<td>6.12</td>
</tr>
<tr>
<td>MT-54</td>
<td>0.073 ± 0.040</td>
<td>0.11 ± 0.05</td>
<td>0.37 ±</td>
<td>0.40 ±</td>
<td></td>
<td></td>
<td>5.28</td>
</tr>
</tbody>
</table>

| 2-ME      | 84.9 |                      |                      |                      | > 256               | > 256               | 3.63      |

*50% inhibitory concentration.

$^a$Calculated by using Chem Bio Draw version 15.0

$^b$n.d = not detected
Often, a correlation is observed between the receptor binding affinity and cytotoxic activity as exhibited in different cancer cell lines. In the series the activity followed the order Cl > F > OMe > Me. The activity decreases with the increasing electron rich substituents. The results showed that high cLogP values results in decreasing activities. The unpublished compounds from our group LIPCHEP, MT-53 and MT-54 are the analogs with a methoxy group and a sulfonamide group in the 2- and 3-position, respectively, see Figure 9. As noticed, none of the compounds are more potent than MT-54.

![Figure 9: Structure of analogs of 2-ME.](image)

The hormone-dependent cancers have led to the development of a new target to reduce estrogen and androgen concentrations. Thus, the enzymes involved in the synthesis and regulation of active steroids are cancer therapy targets. One such enzyme, steroid sulfatase, hydrolyses dehydroepiandrosterone sulfate to dehydroepiandrosterone in peripheral tissues, which is an androgen that support the development and growth of number of hormone-dependent cancers, including breast cancer. Therefore, inhibition of steroid sulfatase could be a potential drug target for the treatment of hormone-dependent cancer. Some compounds similar to our prepared sulfonamides, have been reported. Among the prepared analogs, some may act as an inhibitor of steroid sulfatase with anti-proliferative activity. Such bioactivities may explain the potent activity of very low micromolar to nanomolar concentrations of sulfa analogs 39a-39d. However, additional testing must be performed.

Moreover, the compounds 38a-38d and 39a-39d are currently tested for their anti-angiogenetic effects. The results will soon be available.
3 Future perspectives

In recent years there has been significant investment in the development of potent analogs of 2-ME. Due to its low bioavailability and rapid metabolic degradation, several analogs with anti-proliferative activity containing sulfonamide substituent in the 3-position on the A-ring, which lower its hydrophobic character, have been developed. Some analogs containing cyclic sulfonamide group in the 3-position (see Figure 10) should be synthesized to complete the library.

![Suggested analogs of 2-ME](image)

Figure 10: Suggested analogs of 2-ME

Furthermore, the new analogs reported herein, share some similarities with the two approved drugs Abiraterone (41) and Galeterone (42).

Many important drugs contain the sulfonamide group, including thiazide diuretics. Two widely used examples are sildenafil (43) and bendroflumethiazide (44), see Figure 11. Sildenafil (43) contains an aromatic sulfonamide group. Bendroflumethiazide (44) contains also an aromatic sulfonamide group, in addition contains a cyclic sulfonamide group. In future, the new analogs of 2-ME will also be tested for inhibition of steroid sulfatase.
In collaboration with a student from the Faculty of Pharmacy in Brussels, Koen Van Gansbeke, we have done more reaction with the reductive alkylation reaction. As shown in Scheme 7, six different substituents were introduced to the 2-position in the A-ring. These analogs of 2-ME will also be tested.

As mentioned previously, administration of 2-ME by oral route is a challenging task due poor \textit{in vivo} bioavailability. The 2-ME nanocrystal dispersion formulation applied in clinical trials has showed better bioavailability than the capsule and provides higher plasma concentration levels. Furthermore, several formulation approaches should be investigated to improve the dissolution of 2-ME, include oral lipid-based formulation and nanocrystal dispersion. Administration of 2-ME directly into the oral cavity could be an attractive route for systemic delivery.
4 Conclusions

Synthesis of new analogs of 2-ME is still of interest. Herein, new 2-ME analogs modified both at the C2- and C17 position have been prepared, via two different methods. Synthesis of 2-ethylestradiol (33) in method B, as opposed to method A, is obtained in a much shorter amount of time. The method A gave 55% yield over the three steps. While, the method B gave a 41-71% yield of the desired 2-ethyl substituted estradiol 33. The convenient one-step preparation of 2-ethylestradiol (33) was exceeded to other steroids.

In total, eight new analogs of 2-ME have been synthesized using Suzuki-Miyaura reactions. The compounds 38a-38d and 39a-39d are currently under biological testing for their anti-angiogenic activity and inhibition of tubulin polymerization in collaboration with the National Research Institute of Chinese Medicine, Taipei, Taiwan and in Leuven, Belgium, by professor Sandra Liekens.

The analogs 39a-39d had good activity towards L1210, CEM, HeLa, HMEC-1, VERO and HT29 cell lines. Notably, almost all of the prepared compounds proved more potent than the lead 2-ME toward the CEM cell line.
5 Appendix

5.1 General methods

Unless noted otherwise, all reagents and solvents were used as purchased without further purification. Thin layer chromatography (TLC) analyses were performed on Merck silica gel 60 F$_{254}$ plates and were visualized with UV light and ceric ammonium molybdate (CAM) statin. Column chromatography was carried out on Merck silica gel 60.

NMR spectra were recorded with a Bruker Avance II 400 MHz NMR spectrometer. The peak patterns are indicated as follows: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; q, quartet. Coupling constants ($J$) are reported in hertz (Hz), while the chemical shift values are reported in parts per million (ppm, $\delta$). The CDCl$_3$ $^1$H and $^{13}$C signals (7.26 and 77.16 ppm, respectively), CD$_3$OD $^1$H and $^{13}$C signals (3.31, 4.78 and 49.2 ppm, respectively) and d$_6$-DMSO $^1$H and $^{13}$C signals (δ 2.50 and δ 39.5 ppm, respectively) were used as the NMR solvents. Mass spectra and HRMS were recorded at 70 eV on Autospec Ultima (Micromass Ltd) using EI as the method of ionization. The melting points were measured with a Stuart Scientific melting point apparatus SMP3. Optical rotations were obtained using a Anton Paar’s Modular Circular Polarimeter MCP 100.
5.2 Experimental procedures

5.2.1 Method A

Synthesis of 2-formylestradiol (31)

17β-Estradiol 17 (2.70 g, 10.0 mmol, 1.00 equiv.) was placed in a flame dried three-necked round-bottomed flask under argon atmosphere and dissolved in dry THF (100 mL). Triethylamine (4.20 mL, 30.0 mmol, 3.00 equiv.), anhydrous MgCl₂ (2.85 g, 30.0 mmol, 3.00 equiv.) and paraformaldehyde (1.20 g, 40.0 mmol, 4.00 equiv.) were added, and the reaction mixture was heated at reflux overnight. The reaction was allowed to cool to room temperature and then quenched with 1 M HCl (50.0 mL). The aqueous phase was extracted with EtOAc (3x50.0 mL) and the combined organic phases were washed with brine. The organic extracted was dried over MgSO₄, filtered and concentrated in vacuo. The compound was purified by column chromatography (silica gel, 30% EtOAc in heptane, Rf = 0.19) to give the pure product in 75% yield (2.24 g), m.p. 231-233 °C. ¹H NMR (400 MHz, CDCl₃) δ 10.78 (s, 1H), 9.81 (s, 1H), 7.43 (s, 1H), 6.70 (s, 1H), 3.75 (t, J = 8.5 Hz, 1H), 2.96 – 2.83 (m, 2H), 2.40 – 2.27 (m, 1H), 2.23 – 2.07 (m, 2H), 2.03 – 1.94 (m, 1H), 1.94 – 1.86 (m, 1H) 1.77 – 1.65 (m, 1H), 1.62 – 1.15 (m, 8H), 0.80 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 196.5, 159.7, 148.5, 133.2, 130.9, 119.4, 117.4, 82.2, 50.5, 43.8, 43.6, 38.9, 36.9, 31.0, 30.6, 27.2, 26.6, 23.5, 11.5.

Isolation and characterization of 4-formylestradiol (40)

The residual product 40 was purified by column chromatography (silica gel, 30% EtOAc in heptane, Rf = 0.22) and obtained in a 2% yield as white solid, m.p. 138-140 °C. ¹H NMR
(400 MHz, CDCl$_3$) $\delta$ 11.98 (s, 1H), 10.38 (s, 1H), 7.49 (d, $J = 8.9$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 1H), 3.74 (t, $J = 8.5$ Hz, 1H), 3.36 – 3.28 (m, 1H), 3.18 – 3.07 (m, 1H), 2.33 – 2.26 (m, 1H), 2.24 – 2.07 (m, 3H), 2.05 – 1.93 (m, 2H), 1.77 – 1.66 (m, 1H), 1.57 – 1.15 (m, 11H), 0.79 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 196.0, 161.9, 140.0, 135.9, 132.1, 118.0, 116.1, 82.2, 50.2, 44.3, 43.6, 38.5, 37.1, 31.1, 27.2, 27.0, 26.0, 23.5, 11.5.

**Synthesis of 2-vinylestradiol (32)**

To a suspension of methyltriphienylphosphonium bromide (9.28 g, 26.0 mmol, 3.00 equiv.) in anhydrous THF (150 mL) at 0 °C under argon, $t$-BuOK (2.92 g, 26.0 mmol, 3.00 equiv.) was added. The mixture was stirred for 15-20 minutes to get yellow solution. 2-Formylestradiol 31 (2.60 g, 8.66 mmol, 1.00 equiv.) was added, and the mixture was stirred at cool temperature overnight. A saturated ammonium chloride solution (30.0 mL) was added and the aqueous phase was extracted with DCM (3x20.0 mL). The combined organic phases were dried over MgSO$_4$, filtered and evaporated in vacuo. The crude product was purified by column chromatography (silica gel, 30% EtOAc in heptane, $R_f = 0.23$) to give the pure product in 79% yield (2.03 g), m.p. 159-161 °C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.29 (s, 1H), 6.89 (dd, $J =$ 17.8, 11.2 Hz, 1H), 6.52 (s, 1H), 5.68 (dd, $J =$ 17.7, 1.4 Hz, 1H), 5.29 (dd, $J =$ 11.2, 1.4 Hz, 1H), 3.74 (t, $J =$ 8.5 Hz, 1H), 2.83 – 2.77 (m, 2H), 2.39 – 2.31 (m, 1H), 2.22 – 2.07 (m, 2H), 2.01 – 1.92 (m, 1H), 1.91 – 1.83 (m, 1H), 1.75 – 1.65 (m, 1H), 1.60 – 1.14 (m, 9H), 0.79 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 151.2, 138.3, 133.2, 132.4, 124.7, 122.6, 116.2, 115.2, 82.4, 50.5, 44.3, 43.7, 39.3, 37.1, 31.0, 29.8, 27.6, 26.8, 23.6, 11.5.
Synthesis of 2-ethylestradiol (33)

To a stirred solution of Pd-C (10% (w/w) in EtOAc at room temperature, a solution of 2-vinylestradiol 32 (2.00 g, 6.70 mmol) in EtOAc (16 mL) was added. The reaction mixture was stirred under H₂ gas overnight. Upon completion, DCM (20.0 mL) was added and the mixture was filtered through celite, washed with DCM (3x30.0 mL) and concentrated. The crude product was purified by column chromatography (silica gel, 30% EtOAc in heptane, Rᵣ = 0.30) to give the pure product in 71% yield (1.65 g), m.p. 165-166 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.06 (s, 1H), 6.50 (s, 1H), 4.63 (s, 1H), 3.74 (t, J = 8.5 Hz, 1H), 2.87 – 2.71 (m, 2H), 2.60 (q, J = 7.6 Hz, 2H), 2.39 – 2.29 (m, 1H), 2.23 – 2.04 (m, 2H), 1.99 – 1.92 (m, 1H), 1.90 – 1.82 (m, 1H), 1.75 – 1.65 (m, 1H), 1.56 – 1.26 (m, 7H), 1.23 (t, J = 7.6 Hz, 3H), 0.79 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 151.6, 135.9, 133.0, 127.6, 126.8, 115.6, 82.4, 50.5, 44.4, 43.7, 39.4, 37.2, 31.0, 29.6, 27.7, 26.8, 23.6, 23.4, 14.8, 11.5.

5.2.2 Method B

Reductive alkylation of estradiol (17)

A solution of 17β-estradiol 17 (2.16 g, 8.00 mmol, 1.00 equiv.), Cu(OTf)₂ (7.0 mg, 0.20 mmol, 0.025 equiv.), ethanethiol (3.44 mL, 48.0 mmol, 6.00 equiv.), acetaldehyde (1.34 mL,
24.0 mmol, 3.00 equiv.) and 2,2,2-trifluoretanol (24.0 mL, 3.00 mL/mmol equiv.) was placed in a flame dried round bottomed flask stirred under argon atmosphere and heated under reflux at 50 °C overnight. Then triethylsilane (3.84 mL, 24.0 mmol, 3.00 equiv.) was added and the reaction was further stirred at 50 °C for 3-5 hours, and monitored by TLC (hexane/EtOAC, 8:2). The residual product was purified by column chromatography (silica gel, 30% EtOAc in heptane, R = 0.28) to give the pure product in 52% yield 1.23g, m.p. 165-166 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 7.06 (s, 1H), 6.50 (s, 1H), 3.75 (t, \(J = 8.5\) Hz, 1H), 2.87 – 2.73 (m, 2H), 2.61 (q, \(J = 7.5\) Hz, 2H), 2.39 – 2.29 (m, 1H), 2.22 – 2.08 (m, 2H), 1.98-1.90 (m, 1H), 1.90 – 1.82 (m, 1H), 1.76 – 1.64 (m, 1H), 1.58 – 1.26 (m, 7H), 1.23 (t, \(J = 7.6\) Hz, 3H), 0.79 (s, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 151.6, 135.8, 132.9, 127.7, 126.7, 115.6, 82.4, 50.5, 44.4, 43.7, 39.4, 37.2, 31.0, 29.6, 27.7, 26.8, 23.6, 23.4, 14.8, 11.5.

5.2.3 Synthesis of 2-ethylestrone (34)\(^{55}\)

![Diagram](image_url)

2-Ethylestradiol 33 (1.60 g, 5.30 mmol, 1 equiv.) was dissolved in toluene (53 mL) in a flame dried 250 mL two-necked round bottomed flask equipped with a flame dried Dean-Stark trap and a reflux condenser. Aluminum-isopropoxide (5.41 g, 26.5 mmol, 5.00 equiv.) and cyclohexanone (22.1 mL, 212 mmol, 40.0 equiv.) were added and the reaction mixture was heated at reflux (145 °C) overnight. The reaction mixture was cooled to room temperature and then water (70.0 mL) and 1 M HCl (30.0 mL) were added. The mixture was extracted with EtOAc (3x120 mL), and the aqueous emulsion was acidified with 1 M HCl (50.0 mL) until the emulsion separated. The aqueous layer was extracted with EtOAc (1x100 mL). The combined organic phases were dried over MgSO4, filtered and concentrated in \textit{vacuo}. The residue was triturated with hexane and evaporated in \textit{vacuo}. The crude product was purified by column chromatography (silica gel, 20% EtOAc in heptane, R = 0.21) to give the pure product in 55% yield (880 mg), m.p. 201-204 °C. \(^1\)H NMR (400 MHz, CDCl3) δ 7.03 (s, 1H), 6.52 (s, 1H), 2.87 – 2.75 (m, 2H), 2.60 (q, \(J = 7.5\) Hz, 2H), 2.54 – 2.45 (m, 1H), 2.45 – 2.37 (m, 1H), 2.27 – 2.17 (m, 1H), 2.17 – 2.08 (m, 1H), 2.08 – 1.84 (m, 3H), 1.79 – 1.24 (m, 6H),
1.21 (t, \(J = 7.5\) Hz, 1H) 0.90 (s, 3H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 222.0, 152.2, 135.3, 131.7, 128.1, 126.6, 115.6, 50.8, 48.5, 44.4, 38.8, 36.3, 32.0, 29.5, 27.0, 26.4, 23.4, 21.9, 14.8, 14.2.

### 5.2.4 Synthesis of 3-\textit{ tert}-butyldimethysiloxo-2-ethylestrone (35)\textsuperscript{55}

![Synthesis of 3-\textit{ tert}-butyldimethysiloxo-2-ethylestrone (35)](image)

2-Ethylestrone 34 (870 mg, 2.93 mmol, 1.00 equiv.) was placed in a flame dried 50 mL round bottomed flask and dissolved in dry DMF (15 mL). Imidazole (0.50 g, 7.33 mmol, 2.50 equiv.) and TBSCl (0.66 g, 4.40 mmol, and 1.50 equiv.) were added. The reaction mixture was stirred at room temperature under argon atmosphere for 4 hours and monitored by TLC (heptane: EtOAc, 8:2). A saturated aqueous NaCl (20.0 mL) was added and the aqueous phase was extracted with EtOAc (3x20.0 mL). The combined organic phases were washed with brine (10.0 mL), aqueous AcOH (10%, 10 mL) and NaHCO\(_3\) (10.0 mL). Then it was dried over MgSO\(_4\) and concentrated. The product was purified by column chromatography (silica gel, 20% EtOAc in heptane, \(R_f = 0.47\)) to give the pure product as colorless oil in 54% yield (660 mg). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.06 (s, 1H), 6.50 (s, 1H), 2.92 – 2.79 (m, 2H), 2.57 (q, \(J = 7.6\) Hz, 2H), 2.52 – 2.46 (m, 1H), 2.45 – 2.36 (m, 1H), 2.24 – 2.20 (m, 1H), 2.19 – 2.10 (m, 1H), 2.10 – 1.93 (m, 3H), 1.66 – 1.37 (m, 6H), 1.17 (t, \(J = 7.5\) Hz, 3H), 1.01 (s, 9H), 0.91 (s, 3H), 0.23 (s, 6H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 221.4, 151.8, 135.0, 132.5, 132.4, 126.6, 118.7, 50.9, 48.5, 44.6, 38.9, 36.3, 32.1, 29.7, 27.1, 26.2, 24.0, 22.0, 18.7, 15.1, 14.3, -3.6, -3.6.

### 5.2.5 Synthesis of \((8R,9S,13S,14S)-3-((\textit{ tert}-butyldimethylsilyloxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6\textit{ H}-cyclopenta[\textit{ a}]phenanthren}-17-yl trifluoromethanesulfonate (36)\textsuperscript{55}

55
TBS-protected 2-ethylestrone 35 (580 mg, 1.42 mmol, 1.00 equiv.) and N-Phenylbis(trifluromethanesulfonimide) (760 mg, 2.13 mmol, 1.50 equiv.) were placed in flame dried 50 mL round bottomed flask and dissolved in dry THF (35.0 mL). The reaction mixture was cooled to -78 °C. KHMDS, 0.50 M in toluene (8.50 mL, 4.26 mmol, 3.00 equiv.) was added dropwise and the reaction mixture was stirred at -78 °C for 3 hours. The reaction mixture was then brought to room temperature, quenched with saturated aqueous NH₄Cl (20.0 mL) and extracted with DCM (2x15.0 mL). The combined organic phases were washed with water and brine, dried over MgSO₄ and evaporated in vacuo. The compound was purified by column chromatography (silica gel, 20% EtOAc in heptane, Rf = 0.77) to give the pure product as colorless oil in 64% yield (500 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.04 (s, 1H), 6.51 (s, 1H), 5.63 (dd, J = 3.3, 1.7 Hz, 1H), 2.93 – 2.77 (m, 2H), 2.59 (q, J = 7.5 Hz, 2H), 2.49 – 2.27 (m, 3H), 2.16 – 2.06 (m, 1H), 1.97 – 1.87 (m, 2H), 1.86 – 1.76 (m, 1H), 1.72 – 1.52 (m, 3H), 1.50 – 1.36 (m, 1H), 1.19 (t, J = 7.5 Hz, 3H), 1.03 (s, 9H), 1.03 (s, 3H), 0.25 (s, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 159.8, 151.8, 134.9, 132.5, 126.3, 118.7, 114.8, 54.1, 45.6, 44.8, 37.2, 33.2, 29.5, 28.8, 27.3, 26.3, 26.2, 24.0, 18.6, 15.8, 15.1, -3.6, -3.7.

5.2.6 General procedure for the Suzuki cross-coupling and synthesis of (37a-37d)⁵⁵

The TBS protected steroid triflate 36 (0.28-0.37 mmol, 1.00 equiv.), cesium carbonate (2.00 equiv.) and the boronic acid (1.05 equiv.) were placed in flame dried 10 mL round bottomed flask and dissolved in a 1:1 mixture of water and THF (6.00-8.00 mL). Then Pd(PPh₃)₄ (5 mol
% was added and the reaction mixture was stirred at 60 °C under argon atmosphere overnight. The reaction mixture was then brought to room temperature, added brine (10 mL) and extracted with ethyl acetate (4x5.00 mL). The combined organic phases were dried over MgSO₄ and evaporated in vacuo. The residues was purified by column chromatography (silica gel, 10-20% EtOAc in heptane) to obtain the pure products as light yellow oil.

4-((8S,9S,13S,14S)-3-(tert-Butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methylpyridine (37a)

![Chemical Structure](image)

![Chemical Structure](image)

1H NMR (400 MHz, CDCl₃) δ 8.41 (d, J = 5.2, 1H), 7.16 (s, 1H), 7.11 (dd, J = 5.3, 1.6 Hz, 1H), 7.05 (s, 1H), 6.51 (s, 1H), 6.18 – 6.16 (m, 1H), 2.93 – 2.78 (m, 2H), 2.62 – 2.55 (m, 5H), 2.45 – 2.25 (m, 3H), 2.24 – 2.10 (m, 2H), 1.98 – 1.90 (m, 1H), 1.84 – 1.59 (m, 4H), 1.52 – 1.40 (m, 1H), 1.31 – 1.24 (m, 1H), 1.18 (t, J = 7.5 Hz, 3H), 1.08 (s, 3H), 1.02 (s, 9H), 0.24 (d, J = 1.4 Hz 6H). 13C NMR (101 MHz, CDCl₃) δ 158.5, 153.4, 151.7, 149.2, 145.5, 135.1, 133.0, 132.4, 131.5, 126.3, 121.1, 118.8, 118.7, 57.3, 48.0, 44.6, 37.7, 35.8, 32.0, 29.7, 28.3, 26.9, 26.2, 24.9, 23.1, 18.7, 17.2, 15.1, -3.6, -3.6. Eluent 20% EtOAc in heptane, Rₜ = 0.15, yield 75 mg, 53%, product light yellow oil.

4-((8S,9S,13S,14S)-3-(tert-Butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-fluoropyridine (37b)
1H NMR (400 MHz, CDCl₃) δ 8.13 (d, J = 5.3 Hz, 2H), 7.20 – 7.17 (m, 1H), 7.06 (s, 1H), 6.92 (s, 1H), 6.52 (s, 1H), 6.28 – 6.25 (m, 1H), 2.96 – 2.76 (m, 2H), 2.60 (q, J = 7.4 Hz, 2H), 2.47 – 2.26 (m, 3H), 2.25 – 2.12 (m, 2H), 2.00 – 1.91 (m, 1H), 1.86 – 1.77 (m, 1H), 1.74 – 1.62 (m, 3H), 1.53 – 1.43 (m, 1H), 1.35 – 1.25 (m, 4H), 1.20 (t, J = 7.5 Hz, 3H), 1.09 (s, 3H), 1.03 (s, 9H), 0.90 (t, J = 6.9 Hz, 2H), 0.25 (d, J = 1.5 Hz, 6H). 13C NMR (101 MHz, CDCl₃) δ 165.8, 163.5, 152.4, 152.4, 151.7, 150.6, 150.5, 147.7, 147.6, 135.0, 133.2, 132.8, 132.4, 126.3, 119.4, 119.4, 118.7, 106.9, 106.5, 57.2, 48.0, 44.6, 37.7, 35.7, 32.3, 32.0, 29.7, 29.47, 28.2, 26.9, 26.2, 24.0, 23.1, 18.6, 17.2, 15.1, 14.5, -3.6, -3.7. Eluent 10% EtOAc in heptane, Rf = 0.35, yield 118 mg, 86%, product light yellow oil.

4-((8S,9S,13S,14S)-3-((tert-Butyldimethylsilyloxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methoxypyridine (37c)
1.40 (m, 1H), 1.18 (t, J = 7.5 Hz, 3H), 1.06 (s, 3H), 1.02 (s, 9H), 0.24 (s, J = 1.3 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 164.8, 153.2, 151.7, 148.0, 146.6, 135.1, 133.0, 132.4, 131.6, 126.3, 118.7, 115.6, 108.2, 57.2, 53.9, 48.0, 44.6, 37.7, 35.8, 31.9, 29.7, 28.3, 26.9, 26.2, 24.0, 18.7, 17.2, 15.1, -3.6, -3.6. Eluent 10% EtOAc in heptane, $R_f = 0.38$, yield 113 mg, 65%, product light yellow oil.

4-((8S,9S,13S,14S)-3-((tert-Butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-chloropyridine (37d)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.30 (d, J = 5.2 Hz, 1H), 7.33 (s, 1H), 7.22 (dd, J = 5.3, 1.5 Hz, 1H), 7.05 (s, 1H), 6.51 (s, 1H), 6.26 – 2.23 (m, 1H), 2.95 – 2.76 (m, 2H), 2.59 (q, J = 7.5 Hz, 2H), 2.48 – 2.25 (m, 3H), 2.24 – 2.10 (m, 2H), 1.97 – 1.89 (m, 1H), 1.85 – 1.74 (m, 1H), 1.74 – 1.59 (m, 1H), 1.53 – 1.40 (m, 1H), 1.18 (t, J = 7.5 Hz, 3H), 1.07 (s, 3H), 1.02 (s, 9H), 0.24 (d, J = 1.4 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 152.2, 152.1, 151.7, 149.7, 148.2, 135.0, 133.5, 132.8, 132.4, 126.3, 121.8, 120.3, 118.7, 57.2, 48.1, 44.5, 37.7, 35.7, 32.1, 29.7, 28.2, 26.9, 26.2, 24.0, 18.7, 17.2, 15.1, -3.6, -3.6. Eluent 20% EtOAc in heptane, $R_f = 0.30$, yield 137 mg, 74%, product light yellow oil.

5.2.7 General procedure for the deprotection of (37a-37d)$^{55}$
The TBS protected steroids 37a-37d (0.05-0.20 mmol, 1 equiv.) were placed in flame dried 10 mL round bottomed flask and dissolved in dry THF (2.00 mL). *Tert*-butylammoniumfluoride (1 M in THF, 1.10 equiv.) was added dropwise. The reaction mixture was stirred at room temperature under argon atmosphere overnight. The reaction mixture was poured into saturated aqueous NaHCO$_3$ (2.00-5.00 mL) and extracted with ethyl acetate (4x3.00 mL). The combined organic extracts were dried over MgSO$_4$ and evaporated in *vacuo*. The residues was purified by column chromatography (silica gel, 20-30% EtOAc in heptane) to give the pure products as colorless solids.

(8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a)

\[
\begin{align*}
\text{TBSO} & \quad \text{37a} \quad \text{TBAF} \quad \text{THF, rt.} \\
& \quad \text{88%} \\
\end{align*}
\]

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.41 (d, $J$ = 5.2 Hz, 1H), 7.17 (s, 1H), 7.12 (dd, $J$ = 5.2, 1.7 Hz, 1H), 7.06 (s, 1H), 6.55 (s, 1H), 6.30 (s, 1H), 6.18 – 6.16 (m, 1H), 2.84 (m, 2H), 2.64 (q, $J$ = 7.6 Hz, 2H), 2.57 (s, 3H), 2.47 – 2.25 (m, 3H), 2.25 – 2.09 (m, 2H), 1.97 – 1.89 (m, 1H), 1.84 – 1.61 (m, 5H), 1.52 – 1.36 (m, 1H), 1.24 (t, $J$ = 7.6 Hz, 3H), 1.08 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 158.5, 153.3, 152.3, 149.1, 145.6, 135.6, 132.5, 131.5, 128.0, 126.4, 121.3, 118.9, 115.7, 57.2, 48.0, 44.6, 37.8, 35.8, 32.0, 29.6, 28.2, 27.0, 24.7, 23.6, 17.2, 14.9. Eluent 30% EtOAc in heptane, $R_f$ = 0.18, $[\alpha]_D^{20}$ = +6.7 (c = 0.9, CHCl$_3$), yield 43 mg, 88%, product white solid, m.p. 229-231 °C. HRMS calcd. for C$_{26}$H$_{31}$NO [M]$^+$ 373.2406. Found 373.2403.
(8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b)

\[
\text{\footnotesize \begin{align*}
\text{\( ^1\)H NMR (400 MHz, CDCl}_3) & \delta 8.13 (d, J = 5.3 \text{ Hz, 1H}), 7.18 \text{ (m, 1H), 7.05 (s, 1H), 6.92 (s, 1H), 6.54 (s, 1H), 6.28 – 6.24 (m, 1H), 4.79 (s, 1H), 2.94 – 2.78 (m, 2H), 2.62 (q, J = 7.5 \text{ Hz, 2H}), 2.48 – 2.25 (m, 3H), 2.24 – 2.12 (m, 2H), 1.98 – 1.90 (m, 1H), 1.85 – 1.76 (m, 1H), 1.72 – 1.61 \text{ (m, 3H), 1.52 – 1.40 (m, 2H), 1.24 (t, J = 7.5 \text{ Hz, 3H), 1.08 (s, 3H).} \\
\text{\( ^{13}\)C NMR (101 MHz, CDCl}_3) & \delta 165.8, 163.5, 152.4, 152.3, 151.8, 150.7, 150.6, 147.7, 147.5, 135.7, 133.3, 132.8, 127.7, 126.4, 119.5, 119.4, 115.7, 106.9, 106.6, 57.1. 48.0, 44.5, 37.7, 35.7, 32.0, 29.5, 28.1, 27.0, 23.5, 17.2, 14.8. Eluent 30% EtOAc in heptane, \text{Rf} = 0.25, [\alpha]_{D}^{20} = +19.2 (c = 1.0, \text{ CHCl}_3), \text{yield 48 mg, 64%}, \text{product white solid, m.p. 217-219 °C. HRMS calcd. for C}_{25}\text{H}_{28}\text{FNO} [M]+ 377.2155. Found 377.2143.}
\end{align*}}
\]

(8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38c)

\[
\text{\( ^1\)H NMR (400 MHz, CDCl}_3) \delta 8.08 (d, J = 5.5 \text{ Hz, 1H}), 7.05 \text{ (s, 1H), 6.91 (dd, J = 5.5, 1.5 Hz, 1H), 6.76 (s, 1H), 6.52 (s, 1H), 6.20 – 6.14 (m, 1H), 4.85 (s, 1H), 3.96 (s, 3H), 2.93 – 2.77 (m, 1H).}
\]

61
2H), 2.62 (q, $J = 7.5$ Hz, 2H), 2.42 – 2.09 (m, 5H), 1.97 – 1.89 (m, 1H), 182 – 1.74 (m, 1H), 1.70 – 1.60 (m, 3H), 1.51 – 1.38 (m, 1H), 1.24 (t, $J = 7.5$ Hz, 3H), 1.06 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 164.9, 153.1, 151.7, 147.9, 146.6, 135.8, 133.0, 131.6, 127.7, 126.5, 115.7, 115.6, 108.2, 57.2, 54.0, 48.0, 44.5, 37.7, 35.7, 32.3, 31.9, 29.5, 28.2, 27.0, 23.5, 17.2, 14.9. Eluent 20% EtOAc in heptane, $R_f$ = 0.29, yield 37 mg, 60%, product white solid, m.p. 142-144 °C. HRMS calcd. for C$_{26}$H$_{32}$NO$_2$ [M]•$^+$ 389.2355. Found 389.2344.

(8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d)

\[ TBAF \quad \text{THF, rt.} \quad 45\% \]

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.29 (d, $J = 5.2$, 1H), 7.35 – 7.30 (m, 1H), 7.22 (dd, $J = 5.2$, 1.5 Hz, 1H), 7.04 (s, 1H), 6.54 (s, 1H), 6.29 – 6.22 (m, 1H), 4.82 (s, 1H), 2.90 – 2.78 (m, 2H), 2.62 (q, $J = 7.5$ Hz, 2H), 2.45 – 2.25 (m, 3H), 2.23 – 2.12 (m, 2H), 2.01 – 1.88 (m, 1H), 1.84 – 1.75 (m, 1H), 1.71 – 1.61 (m, 3H), 1.51 – 1.41 (m, 1H), 1.24 (t, $J = 7.5$ Hz, 3H), 1.07 (s, 3H). Eluent 20% EtOAc in heptane, $R_f$ = 0.18, [α]$^D_{20}$ = +6.0 (c = 0.5, CH$_2$Cl$_2$), yield 9 mg, 45%, product white solid, m.p. 238-240 °C. HRMS calcd. for C$_{25}$H$_{28}$ClNO [M]•$^+$ 393.1859. Found 393.1855.

5.2.8 General procedure for the synthesis of sulfonamides (39a-39d)$^{35}$
The estrogens (38a-38d, 0.06-0.33 mmol, 1.00 equiv.) and 2,6-di-tert-butyl-4-methylpyridine (DBMP, 3.00 equiv.) were placed in flame dried 10 mL round bottomed flask and dissolved in dry dichloromethane (3-5 mL) under argon atmosphere. The reaction mixture was cooled to 0 °C before sulfamoyl chloride (2.95 equiv.) was added and stirred for additional 30 minutes. Then the reaction mixture was stirred at room temperature overnight. The reaction mixture was added saturated aqueous NaHCO$_3$ (5 mL) and extracted with ethyl acetate (3x10 mL). The combined organic extracts were dried over MgSO$_4$ and evaporated in vacuo. The residues was purified by column chromatography (silica gel, 30-50% EtOAc in heptane) to yield the pure products as colorless solids.

(8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39a)

![Chemical structure](image)

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.39 (d, $J = 5.3$ Hz, 1H), 7.18 (m, 2H), 7.14 (m, 1H), 7.12 (s, 1H), 6.22 (t, $J = 2.5$ Hz, 1H), 5.16 (s, 2H), 2.93 – 2.87 (m, 2H), 2.71 (q, $J = 7.5$ Hz, 2H), 2.59 (s, 3H), 2.45 – 2.28 (m, 3H), 2.25 – 2.12 (m, 2H), 2.01 – 1.93 (m, 1H), 1.83 – 1.60 (m, 5H), 1.52 – 1.40 (m, 1H), 1.24 (t, $J = 7.6$ Hz, 3H), 1.08 (s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 157.5, 152.5, 146.2, 139.2, 135.8, 133.8, 133.6, 126.6, 121.5, 120.9, 118.5, 56.7, 47.4, 44.2, 36.8, 35.2, 31.5, 29.0, 27.4, 26.3, 23.9, 23.0, 16.7, 14.6. Eluent 50% EtOAc in heptane, $R_t = 0.16$. $[^{20}]D^2 = +15.5$ (c = 0.6, CH$_2$Cl$_2$), yield 15 mg, 42%, product white solid, m.p. 180-182 °C. HRMS calcd. for C$_{26}$H$_{32}$N$_2$O$_3$S [M]$^+$ 452.2134. Found 452.2131.
(8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[α]phenanthren-3-yl sulfamate (39b)

![Chemical structure of 38b](image)

H$_2$NSO$_2$Cl, DBMP, CH$_2$Cl$_2$

DCM, 0 °C - rt.

81%

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.12 (d, $J$ = 5.5 Hz, 1H), 7.17 (m, 2H), 7.11 (s, 1H), 6.91 (s, 1H), 6.31 – 6.23 (m, 1H), 5.05 (s, 2H), 2.97 – 2.86 (m, 2H), 2.71 (q, $J$ = 7.5 Hz, 2H), 2.48 – 2.11 (m, 5H), 2.08 – 1.92 (m, 1H), 1.87 – 1.60 (m, 5H), 1.55 – 1.39 (m, 1H), 1.23 (t, $J$ = 7.5, 3H), 1.08 (s, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 165.4, 163.4, 152.2, 152.2, 150.6, 150.5, 147.7, 147.6, 146.7, 139.7, 136.2, 134.2, 133.3, 127.1, 121.9, 119.5, 119.4, 106.9, 106.5, 57.1, 47.9, 44.7, 37.2, 35.6, 32.0, 29.4, 27.9, 26.7, 23.5, 17.1, 15.0.

Eluent 50% EtOAc in heptane, $R_f$ = 0.57, $[α]_{D}^{20}$ = +24.4 (c = 0.84, CH$_2$Cl$_2$), yield 39 mg, 81%, product white solid, m.p. 187-189 °C. HRMS calcd. for C$_{25}$H$_{29}$FN$_2$O$_3$S $[M]^{+}$ 456.1883. Found 456.1865.

(8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[α]phenanthren-3-yl sulfamate (39c)

![Chemical structure of 38c](image)

$\text{H}_2\text{NSO}_2\text{Cl, DBMP, CH}_2\text{Cl}_2$

DCM, 0 °C - rt.

90%

$^1$H NMR (400 MHz, DMSO) δ 8.09 (d, $J$ = 5.4 Hz, 1H), 7.93 (s, 2H), 7.19 (s, 1H), 7.03 (s, 1H), 7.02 (dd, $J$ = 5.4, 1.5 Hz, 1H), 6.75 (s, 1H), 6.34 – 6.29 (m, 1H), 3.85 (s, 3H), 2.88 – 2.78 (m, 2H), 2.69 – 2.57 (m, 2H), 2.46 – 2.37 (m, 1H), 2.36 – 2.06 (m, 4H), 1.94 – 1.86 (m,
1H), 1.77 – 1.51 (m, 4H), 1.48 – 1.33 (m, 1H), 1.13 (t, \( J = 7.5 \) Hz, 3H), 1.02 (s, 3H). \(^{13}\)C NMR (101 MHz, DMSO) \( \delta \) 163.9, 151.7, 146.7, 146.5, 146.1, 138.0, 134.9, 133.4, 131.5, 126.1, 121.6, 114.8, 106.7, 56.2, 53.0, 46.8, 43.6, 36.4, 34.6, 30.9, 28.4, 26.9, 25.9, 22.4, 16.3, 14.7. Eluent 30\% EtOAc in heptane, \( R_f = 0.22, [\alpha]_{D}^{20} = +9.0 \) (c = 1.0, C\(_2\)H\(_6\)OS), yield 27 mg, 90\%, product white solid, m.p. 197-200 °C. HRMS calcd. for C\(_{26}\)H\(_{32}\)N\(_2\)O\(_4\)S [M]•\(^{+}\) 468.2083. Found 468.2075.

(8\(S\),9\(S\),13\(S\),14\(S\))-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6\(H\)-cyclopenta[a]phenanthren-3-yl sulfamate (39d)

\(^{1}\)H NMR (400 MHz, MeOD) \( \delta \) 8.31 (d, \( J = 5.3 \) Hz, 1H), 7.51 (s, 1H), 7.46 (dd, \( J = 5.2, 1.6 \) Hz, 1H), 7.25 (s, 1H), 7.14 (s, 1H), 6.47 -6.44 (m, 1H), 2.99 – 2.91 (m, 2H), 2.76 (q, \( J = 7.5 \) Hz, 2H), 2.56 – 2.20 (m, 6H), 2.08 – 2.00 (m, 1H), 1.92 – 1.82 (m, 1H), 1.78 – 1.67 (m, 3H), 1.60 – 1.44 (m, 1H), 1.25 (t, \( J = 7.5 \) Hz, 3H), 1.16 (s, 3H). \(^{13}\)C NMR (101 MHz, MeOD) \( \delta \) 152.8, 152.4, 150.3, 149.8, 148.0, 139.7, 136.6, 135.2, 135.0, 127.4, 122.8, 122.5, 121.5, 58.0, 45.5, 38.4, 36.3, 32.5, 29.9, 28.6, 27.5, 24.0, 17.0, 15.1. Eluent 50\% EtOAc in heptane, \( R_f = 0.54, [\alpha]_{D}^{20} = +20.5 \) (c = 0.8, CH\(_2\)Cl\(_2\)), yield 75 mg, 81\%, product white solid, m.p. 190-192 °C. HRMS calcd. for C\(_{25}\)H\(_{29}\)ClN\(_2\)O\(_3\)S [M]•\(^{+}\) 472.1587. Found 472.1579.
6 References

Accessed January 21\textsuperscript{st}, 2016


(4) Skeel, R. T. \textit{Handbook of Cancer Chemotherapy}; 7\textsuperscript{th} ed.; Lippincott Williams and Wilkins, \textbf{2007}, 1-32.


(10) Sherbet, G. V. \textit{Anticancer Research} \textbf{2015}, \textit{35}, 5767-5772.


(42) Chua, Y. S.; Chua, Y. L.; Hagen, T. Molecular Cancer Therapeutics 2010, 9, 224-235.


7 Appendix

7.1 $^1$H NMR and $^{13}$C NMR spectra of the synthesized compounds
7.1.1 2-Formylestradiol (31)
7.1.2 4-Formylestradiol (40)
7.1.3 2-Vinylestradiol (32)
7.1.4 2-Ethylestradiol (33) (via method A)
7.1.5 2-Ethylestradiol (33) (via method B)
7.1.6 2-Ethylestrone (34)
7.1.7 3-tert-Butyldimethylsiloxy-2-ethylestrone (35)
7.1.8 \((8R,9S,13S,14S)-3-((\text{tert}-\text{butyldimethyl}silyl)oxy)-2\text{-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6}H\text{-cyclopenta}\[a\]\text{phenanthren-17-yl trimethane-sulfonate (36)}}
7.1.9 4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methylpyridine (37a)
7.1.104-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-fluoropyridine (37b)
7.1.114-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-methoxypyridine (37c)
7.1.124-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)-2-chloropyridine (37d)
7.1.13(8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a)
7.1.14(8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b)
7.1.15(8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38c)
7.1.16(8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d)
7.1.17(8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39a)
7.1.18(8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-
7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl
sulfamate (39b)
7.1.19(8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39c)
7.1.20(8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl sulfamate (39d)
7.2 HRMS of synthesized compounds

7.2.1 (8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38a)
(8S,9S,13S,14S)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38b)
7.2.3 (8S,9S,13S,14S)-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (380)
7.2.4 (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (38d)
(8S,9S,13S,14S)-2-ethyl-13-methyl-17-(2-methylpyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl-sulfamate (39a)
7.2.6 (85,95,135,145)-2-ethyl-17-(2-fluoropyridin-4-yl)-13-methyl-octahydro-6H-cyclopenta[a]phenanthren-3-yl-sulfamate (39b)

mass calc. mass pmm dbp score
data:
max: 200.0
min: 10.0
range: 190.0

12 formula(s) evaluated with results within limits (up to 50 closest results for each mass)

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%
Tolerance = 10.0 ppm / DEB: min = -1.5, max = 50.0

Single Mass Analyzes

Elemental Composition Report
7.8.9.11.12.13.14.15-octahydro-6H-cyclopenta[a]phenanthren-3-yl-sulfamate (39c)

([8S,9S,13S,14S]-2-ethyl-17-(2-methoxypyridin-4-yl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl-sulfamate (39c))

Elemental Composition Report

 Mass Calc. Mass MDA PPK DBE Score Formula

Peak Mass:

Maximum: 468,2075
Minimum: 200,01.0

Tolerance = 10.0 PPM
DBE: min = 1.5, max = 50.0

Isotope Cluster Parameters: Separation = 1.0, Abundance = 1.0%
Monoisotopic Mass: Odd and Even Electron Ions

Vomage ESI+
7.2.8 (8S,9S,13S,14S)-17-(2-chloropyridin-4-yl)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydropyrido[3,4-c]pyridin-4-yl-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ylsulfamate (39d)

Elemental Composition Report

Voltaage: 9.193

MS: 472.172

Molecular Mass: 472.172

Monisotopic: 472.166

Isotope Cluster Parameters: Separation = 1.0, Abundance = 1.0%

Tolerance = 1.0 ppm / DBE: min = -1.5, max = 5.0