A novel approach for ER\(^+\) breast cancer treatment: A new compound that modulates aromatase and ER

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Graphical Abstract
Abstract

Estrogen receptor-positive (ER⁺) breast cancer is the most common subtype of breast cancer worldwide. Estrogens, after being synthetized by aromatase, bind to ERα promoting breast cancer proliferation. Besides the success of the already approved therapies, they induce several side effects, reason why it is crucial to discover novel therapeutic approaches. Considering this, our goal is to discover multi-target compounds able to simultaneously inhibit aromatase and modulate ERα. For that, the known aromatase inhibitors (AIs) and ERα antagonists were collected and chemical descriptors were constructed and organized in clusters. After that, the selected compounds were analyzed by molecular docking. Anti-aromatase activity was evaluated in human placental microsomes. Aromatase and ERα expression was assessed by Western-Blot in ER⁺ an aromatase-overexpressing breast cancer cell line (MCF-7aro).

One compound (MT1) was selected to be studied in microsomes and in MCF-7aro cells. This compound was not able to inhibit aromatase in microsomes, but curiously, MT1 decreased aromatase protein levels in MCF-7aro cells. Furthermore, MT1 impaired ERα activation, acting as an ERα antagonist. This represents a great advantage for breast cancer treatment, since aromatase and ERα are key targets in this type of cancer.

Keywords: ER⁺ Breast Cancer, Aromatase, ERα, Multi-target
Introduction

Breast cancer
2018

New cases/2018: 2,088,849
Deaths/2018: 626,679

The Global Cancer Observatory, March 2019
Introduction

Breast cancer

ER⁺/PR⁺  HER2⁺  Triple Negative

70-80% ER⁺

60% pre-menopausal women  75% post-menopausal women

Introduction

Aromatase (CYP19)

- Belongs to the cytochrome P450 family
- Product of the CYP19A1 gene on chromosome 15
- Highly expressed in the ovaries of pre-menopausal women and in adipose cells of post-menopausal women
- In ER$^+$ breast cancer patients is overexpressed
- Responsible for the conversion of androgens into estrogens

Introduction

Aromatase
**Introduction**

**Estrogen Receptor (ER)**

ERs

- **ERα**
  - Growth and survival of breast epithelial cells, through cell proliferation and inhibition of apoptosis
  - Tumor development

- **ERβ**
  - Anti-proliferative and pro-apoptotic properties
  - Tumor suppression

Introduction

**Endocrine Therapy**

**ER Modulators**

- **Tamoxifen**
  - Pre-menopausal women

- **Fulvestrant**
  - Pre- and post-menopausal women

Introduction

Endocrine Therapy

Aromatase Inhibitors (AIs)

Non-Steroidal

Anastrozole

Letrozole

Steroidal

Exemestane

Post-menopausal women and pre-menopausal women after ovaries ablation

And if we find a compound able to inhibit aromatase and simultaneously modulate ERs activity?
Sequence Alignment

Aromatase  + + + F G I G S C I T L I I M I H Y S S R F G S L I
ERα       + P P I L Y S E S E S A M B M M G L L T N L A D R E L V
ERβ       P P H V L I S F T E A S M M M S L T K L A D K E L V

Aromatase  M G I I F N N W   W Q A L I I I Q C I L E E H M L I A
ERα       H M I L E S A W   N L L L D R Q G K S V E G M V E
ERβ       H M + + + + C W .. + L V L D R E G K C V E G I L E

Aromatase  A P D T M S V   .
ERα       I F D M L L A
ERβ       I F D M L L T

Conservation of important residues
Are the ligands of these targets similar?
Aim

Understand the specificities of each set of compounds that interact with each one of the three targets

Find the common features

Discover a multi-target compound
Results and discussion

\[\begin{align*}
2619 \text{ aromatase inhibitors} &+ \\
3701 \text{ ER}_\alpha \text{ antagonists} &+ \\
665 \text{ ER}_\beta \text{ agonists} & = \\
\text{6985} &
\end{align*}\]

\[\begin{align*}
1210 \text{ aromatase inhibitors} &+ \\
1557 \text{ ER}_\alpha \text{ antagonists} &+ \\
73 \text{ ER}_\beta \text{ agonists} & = \\
\text{2840} &
\end{align*}\]
Results and discussion

**Chemical Descriptors Evaluation – 1D Descriptors**

- The majority of the AIs has a molecular weight between 100 g/mol and 300 g/mol
- ERα antagonists have higher molecular weights

- AlS have volumes mainly between 301 Å³ and 600 Å³
- ERα antagonists have higher molecular volumes
Results and discussion

Chemical Descriptors Evaluation – 1D Descriptors

- The majority of AIs have log P values between 1.0 and 3.0
- ER\(\alpha\) antagonists present log P values between 2.1 and 5.0

- The majority of the compounds have 3 or 4 rings
Results and discussion

Chemical Descriptors Evaluation – 1D Descriptors

- The majority of the compounds have between 2 and 5 donor groups
- More than 60% of AIs do not have any acceptor group
- ERα antagonists have typically 2 acceptor groups
Results and discussion

**Extended-Connectivity Fingerprints – 2D Descriptors**

- Constructed with ChemAxon software
- Represent molecular structures by means of circular atom neighborhoods
- ECFPs are circular topological fingerprints designed for molecular characterization, similarity searching and structure-activity models
- Applied in VS studies

*Source: ChemAxon*
Results and discussion

2840 Compounds

Clusters

Similarity between 0.6 and 0.9

175 clusters

2 clusters containing compounds of the three targets

Clusters composition

<table>
<thead>
<tr>
<th>Cluster ID</th>
<th>ERβ</th>
<th>ERα</th>
<th>Aromatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>49</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Number of compounds
Results and discussion

- The selected compound was designated as MT1
Results and discussion

Anti-aromatase activity of MT1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Anti-aromatase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1 (2 µM)</td>
<td>-2.81 ± 3.05</td>
</tr>
<tr>
<td>Exe (1 µM)</td>
<td>97.86 ± 0.52</td>
</tr>
<tr>
<td>Ana (1 µM)</td>
<td>99.12 ± 0.02</td>
</tr>
<tr>
<td>Let (1 µM)</td>
<td>99.69 ± 0.06</td>
</tr>
</tbody>
</table>

MT1 is not able to inhibit aromatase
MT1 effects on aromatase expression levels in MCF-7aro cells

Western-Blot of aromatase

Aromatase 58 kDa
β-tubulin 55 kDa

MT1 induced a decrease of 44\% on aromatase expression levels
Are the effects induced by MT1 on aromatase expression levels a result of a decreased *CYP19A1* gene expression or a consequence of aromatase degradation?
MT1 effects on CYP19A1 transcription levels in MCF-7aro cells

Results and discussion

MT1 did not induce any change in CYP19A1 transcript levels
Results and discussion

**MT1 effects on ERα activation in MCF-7aro cells**

Western-Blot of ERα phosphorylation at Ser118 and Ser167

- **p-ERα**<sup>S118</sup> (66 kDa)
  - ERα (66 kDa)
  - β-tubulin (55 kDa)

- **p-ERα**<sup>S167</sup> (66 kDa)
  - ERα (66 kDa)
  - β-tubulin (55 kDa)

![Graphs showing phosphorylation levels](image)

**MT1 may act as an ERα antagonist**
Conclusions

- Molecular Descriptors analysis:
  - Aromatase inhibitors and ERα antagonists have similar values of molecular weight, volume, rings and donor groups.
  - The main difference among all the compounds is the number of acceptor groups.

- The compound selected, MT1:
  - Did not inhibit aromatase but induces aromatase degradation
  - Impairs ERα activation, acting as an ERα antagonist

MT1 is able to modulate two key targets of ER+ breast cancer, which represents a great advantage in this type of cancer.
Hit to lead transformation

Alteration of the substituents

Weak compound

Strong compound
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