Synthesis, biological evaluation, and docking study of indole aryl sulfonamides as aromatase inhibitors

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Synthesis, biological evaluation, and docking study of indole aryl sulfonamides as aromatase inhibitors

Graphical Abstract

1-30

3
IC$_{50}$: 0.49 µM

22
IC$_{50}$: 0.75 µM

7
IC$_{50}$: 0.19 µM

23
IC$_{50}$: 0.20 µM
Breast cancer is the most common type of cancer in women, and two-thirds of post-menopausal breast cancer are estrogen-dependent.[1] The estrogen production is regulated by CYP19A1 (aromatase) responsible for the conversion of androgens (C19) to estrogens (C18) by demethylation and aromatization of the steroidal A-ring.[2] Two types of endocrine therapies are available: selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs). AIs suppress the aromatase activity interacting with the substrate-binding site of the aromatase, and, based on their structure, were usually classified in steroidal (exemestane) and non-steroidal (letrozole and anastrozole). The third generation of AIs is the most selective and with less secondary effects compared to the previous generations.[3]

In order to identify new aromatase enzyme inhibitors,[4] a library of thirty aryl sulfonamide derivatives containing an indole nucleus, have been synthesized. All compounds were tested using an enzymatic assay to identify compounds with a good inhibitor activity of CYP19A1, compared to letrozole. The IC$_{50}$ of best ones, cell-viability and cytotoxicity on MCF7 human breast cancer cells were further evaluated. Finally, the docking study showed that the best active compounds efficiently bound in the active site of the aromatase; high values of HBD and low levels of HBA are the principal requirement evidenced by the QSAR model.

**Keywords:** aromatase, breast cancer, aromatase inhibitors, sulfonamide, indole, docking, QSAR

Aromatase is a monooxygenase coded by the gene CYP19 located in chromosome 15q21.

Aromatase is responsible for the conversion of androgens (C19) to estrogens (C18) by demethylation and aromatization of the steroidal A-ring.

The natural substrate of aromatase is androstenedione.
Introduction

- Elevated expression of aromatase is found in post-menopausal estrogen-dependent breast cancer.
- The first line of treatments is the use of antiestrogens and aromatase inhibitors (AIs).
- AIs bind to the substrate-binding site of the aromatase.
- Based on the structure, AIs can be divided in:
  - **Steroidal AIs** (exemestane)
    - strictly related to androstenedione, bind irreversibly to the active site (irreversible inhibition).
  - **Non-steroidal AIs** (letrozole and anastrozole).
    - coordinate to the heme iron of the enzyme in a reversible manner (reversible inhibition).

- The third generation of AIs is potent and specific, with strong effect and well tolerated.
- Side effects include bone loss, joint pain, cardiac events.
- Acquired resistance could be developed during the five years therapy.
**Aim of the work**

- The discovery of non-steroidal sulfonamide-containing AIs is the aim of this work.
- A library of 30 aryl sulfonamide (1-30) is synthesized, the percentage of aromatase inhibition and the \( \text{IC}_{50} \) are valued, cell viability and cytotoxicity are tested, a docking study and QSAR are realized.

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<td>21, 30</td>
<td>0</td>
<td>Cl</td>
<td>F</td>
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</table>
**Results and discussion**

**Synthesis**

Reagents and conditions: 3 eq NEt₃, dry CH₂Cl₂, N₂, 0°C for 2h, r.t. 18h-22h.

- The purification by Liquid Chromatography gave the purified compounds 1-30.
- Melting points were determined.
- ¹H and ¹³C Nuclear Magnetic Resonance spectra were monitored.
- Elemental analyses were carried out.

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Results and discussion

Aromatase Inhibition Studies

- The *in vitro* anti-aromatase activity was valued using a commercial fluorimetric assay kit using letrozole (LTR, IC\textsubscript{50} = 1.9 nM) as reference drug.
- Compounds 1-30 and letrozole were tested at 1 μM to calculate the percentage of aromatase inhibition, and at 5 different concentrations (0-100 μM) for IC\textsubscript{50} calculation.
- Experiments were repeated in triplicate.

\[\text{% of inhibition is shown only for values superior than 10%}.\]

<table>
<thead>
<tr>
<th>Cmp</th>
<th>IC\textsubscript{50} (μM)</th>
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<tr>
<td>3</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>7</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>22</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>23</td>
<td>0.20 ± 0.01</td>
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</table>

\(^{a}\text{The value represents mean of triplicate determinations ±RSD}\)

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Results and discussion
Viability and Cytotoxicity Assay

- The cell viability was assessed by MTT assay on human breast cancer cell line (MCF7) in the range 0-250 µM for 24, 48 and 72 h for the best active compounds 3, 7, 22 and 23.

- A time and dose-dependent decrease with loading concentrations of 3, 7, 22-23 were revealed.
- Cell metabolic activity is assessed at around 40-45% after the exposure to 10 µM at 48 h.
- Compounds 3 and 7 reach values of metabolic activity around 20% at higher doses.

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Results and discussion

Viability and Cytotoxicity Assay

• The cytotoxicity of compounds 3, 7, 22-23 was assessed measuring the LDH (lactate dehydrogenase) released from human breast cancer cell line (MCF7) in the concentration range of 0-250 µM for 24 h.

• There is a secretion of the enzyme assesses at 40% already after 24 hours of exposure, being doubled with respect to the amount of LDH released with DMSO alone.

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Molecular Docking

- The three-dimensional interaction diagrams revealed that 3, 7, 22, and 23 were bound to aromatase enzyme via most of the binding residues of the native substrate androstenedione.
- The fundamental amino acid residues include Phe134, Trp224, Val370, Val373 and Met374 in addition to the cofactor heme group (HEM) which plays an essential role in binding.

3D interaction diagrams representing the docked conformation of best active compounds inside the human placental aromatase. Interactions between binding residues and the ligands are represented by dashed lines and arrows.

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Results and discussion

• The aromatase inhibitory activity of the dataset compounds were predicted using 10 physicochemical properties* as molecular descriptors to build the Multiple Linear Regression (MLR) model.

• A QSAR model was successfully constructed using MLR algorithm:

\[
\text{PIC}_{50} = -0.092\text{aLogP} - 0.42\text{HBA} + 0.41\text{HBD} + 0.36\text{RB} - 0.82\text{HAC} + 0.45\text{RC} - 0.06\text{PSA} + 0.22\text{E-state} + 0.37\text{Polar} - 0.032\text{MR}
\]

The QSAR model demonstrated that high values of hydrogen bond donors (HBD) and low value of hydrogen bond acceptors (HBA) are required for the compounds to be active.

• The atomic contribution maps from KPLS (Kernel-Based Partial Least Squares) QSAR was generated.

• The assessment of favorable and unfavorable structural characteristics revealed the most relevant structural features for the aromatase inhibitor.

*Physicochemical descriptors: octanol-water partition coefficient (aLogP), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), number of rotatable bonds (RB), and heavy atom count (HAC), ring count (RC), polar surface area (PSA), electrotopological state (E-state), molar refractivity (MR), and molecular polarizability (Polar).

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Results and discussion

- The atomic contribution maps of the four compounds showed considerable aromatase inhibitory activity.

![Chemical structures](images)

3, PIC50: 0.313  
7, PIC50: 0.789  
22, PIC50: 0.123  
23, PIC50: 0.699

Positive contributions are depicted in red, negative contributions in blue, white for neutral contributions, the color intensity shows the magnitude of the effect.

- The benzene ring and the sulphonamide moiety contribute positively to the activity.
- The aliphatic hydrocarbon chain linking the sulphonamide moiety to the indole ring in 7 and 22 positively contribute to the activity.
- The methylene group linking the benzene ring to the sulphur in 22 possesses no contribution to the activity in contrast to the methylene group in 7 which showed positive influence.
- The para methyl group in the aromatic ring in 3 has a favorable contribution to the activity.

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Conclusions

• A library of 30 aryl sulfonamide indole derivatives was synthesized by a one-step chemical process starting from commercially available products.

• The capability to inhibit aromatase was evidenced for four compounds (3, 7, 22, and 23) showing IC\textsubscript{50} values in the range of sub-micromolar (0.16-0.75 μM).

• The cell viability and cytotoxicity on MCF7 demonstrated a time- and dose-dependent decrease of active metabolizing cells over the time of the culture.

• The docking study revealed that the best active compounds interact with the key residues in the active site of the enzyme.

• The fundamental characteristics of compounds are the indole nucleus interacting with the heme of aromatase, the sulfonamide for hydrogen bonding, and the presence of an unsubstituted benzene or a para-methylbenzene to interact with a hydrophobic pocket.

• The QSAR model demonstrated that high values of hydrogen bond donors (HBD) and low value of hydrogen bond acceptors (HBA) are required for the compounds to be active.
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