The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
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Novel sulfonamide inhibitors of CYP19A1: design, synthesis, biological assays, and in silico study

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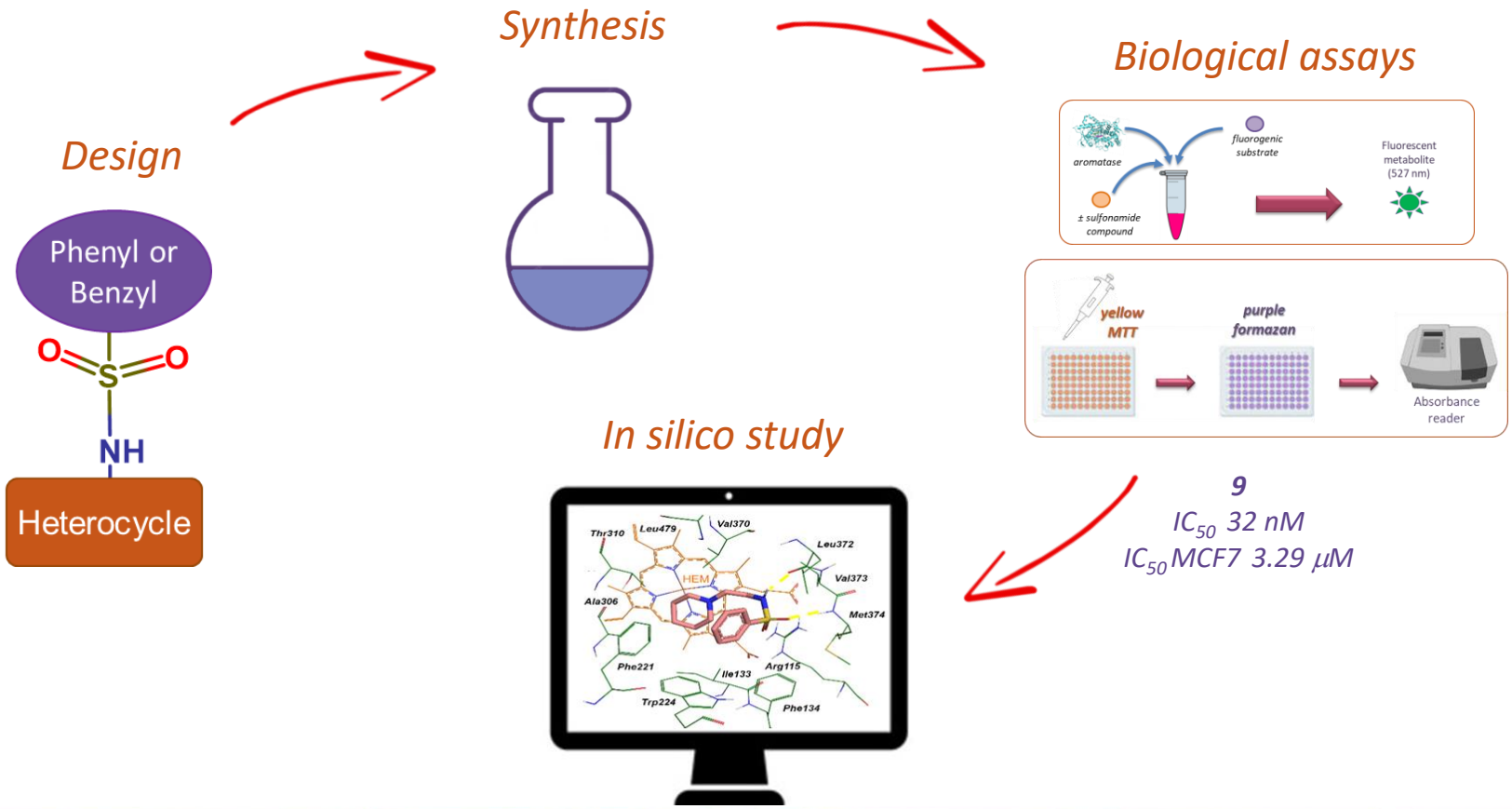
Graphical abstract



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Abstract

Aromatase (CYP19A1) is one of the target enzymes in breast cancer fight. Several aromatase inhibitors (AIs), either steroidal or non-steroidal AI, have been discovered in recent years, however avoiding adverse effects and overcoming resistance mechanism still remain a challenging.

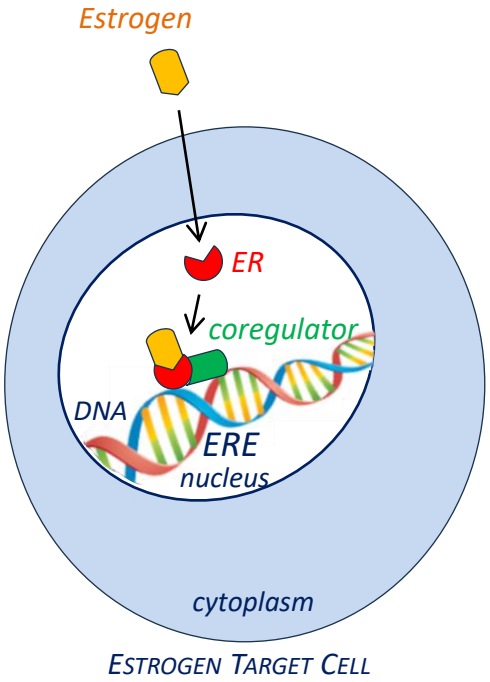
As a continuation of the discovery of novel anti-breast cancer drugs, a library of 17 sulfonamide compounds able to inhibit the aromatase enzyme by the interaction with the HEME group, was designed and synthesized. The general structure of compounds is characterized by the presence of a phenyl or benzyl ring linked to the S atom of the sulfonamide group, while the N atom is substituted with an aromatic or non-aromatic heterocycle. The one-pot synthesis is realized by the reaction between the appropriate amine and the aryl sulfonyl chloride. All compounds were tested for the enzymatic IC_{50}, cellular IC_{50} in MCF7 breast cancer cell line, and for the evaluation of the cell viability (MTT assay using MCF7). To understand the binding mode and finding out the molecular interactions responsible for the effective binding to the active site of aromatase, computational simulations were carried out using Maestro by Schrödinger. Additionally, using QikProp, the physicochemical parameters of drug candidates were calculated in order to examine their pharmacokinetic characteristics.

Keywords: aromatase inhibitor; breast cancer; CYP19A1; *in silico* study; sulfonamide

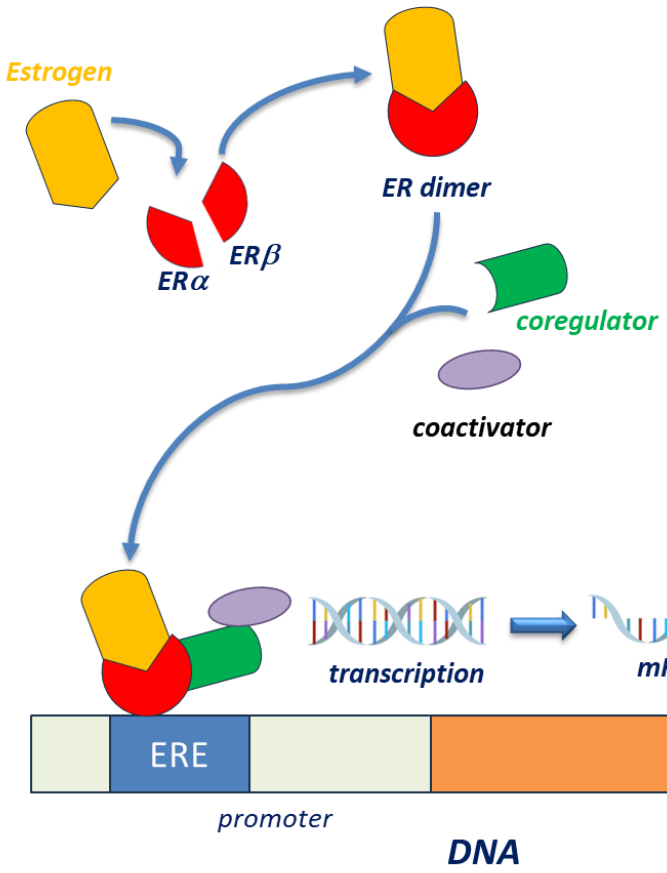
Introduction: Breast cancer

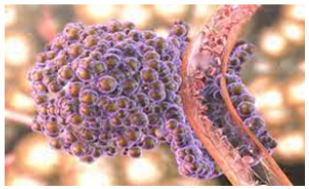
Breast cancer is the most common cancer in women and 2/3 of post-menopausal breast cancer are estrogen-dependent.

- Estrogen target cells (breast, uterine lining, liver, etc.) that contain Estrogen Receptor (ER) in the nucleus.
- After the binding, the estrogen-ER complex binds to specific Estrogen Response Element (ERE) in DNA.
- The recruitment of co-activators, activate the DNA transcription.
- In women with ER+ cancers, the role of estrogen is the fueling of the growth and division of breast cancer cells.
- Therefore, such cancers are susceptible to treatment that modulate the estrogen production.



Introduction: Breast cancer therapy



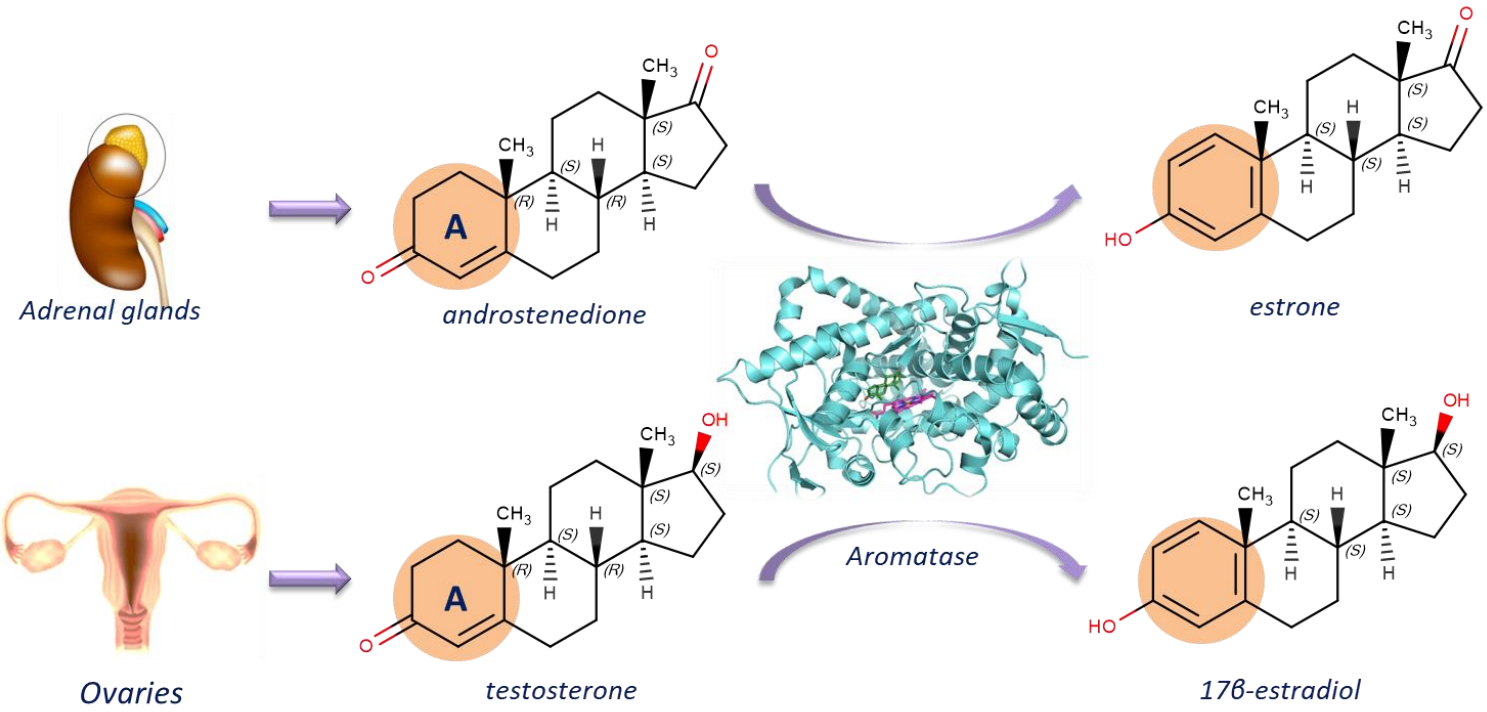


Breast Cancer Cells proliferation

- Selective Estrogen Receptor Modulators (SERMs)
- Selective Estrogen Receptor Down Regulators (SERDs)
- **Aromatase Inhibitors (AIs)**
- Sulphatase Inhibitors (SIs)
- Miscellaneous (AI + SI)

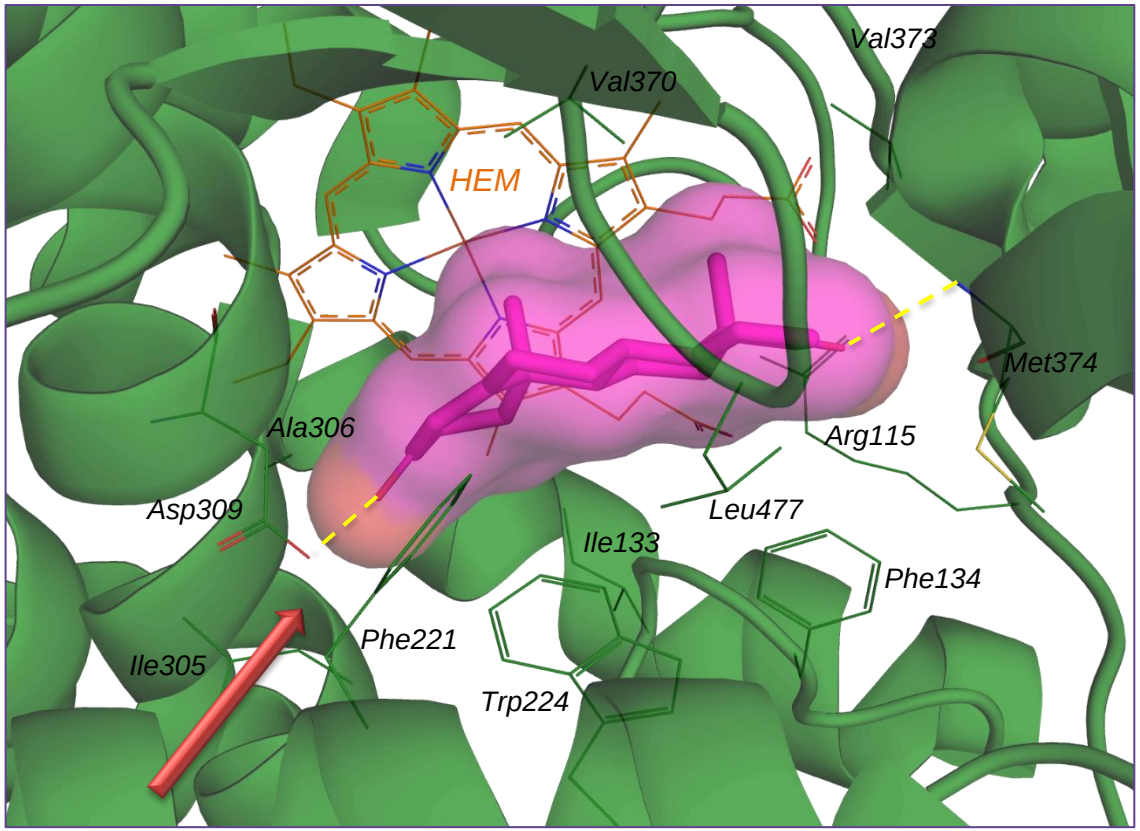
Introduction: Role of Aromatase in Breast cancer

- In Post-menopause, the little amount of estrogen comes from the aromatase action on androstenedione and testosterone produced by adrenal glands and ovaries.



- Aromatase is highly expressed in adipose cells of post-menopausal women.
- Overexpressed in ER^{+} breast cancer patients.

Introduction: Aromatase active site



Access channel

PDB:3EQM

The active site of the aromatase enzyme is shaped like the androgen backbone as represented by the androstenedione surface in magenta.

The semitransparent surface closely resembles the shape, size, and puckering of the steroid backbone.

The only opening to the pocket is the one to the active site access channel indicated by the red arrow.

Introduction: Aromatase inhibitors

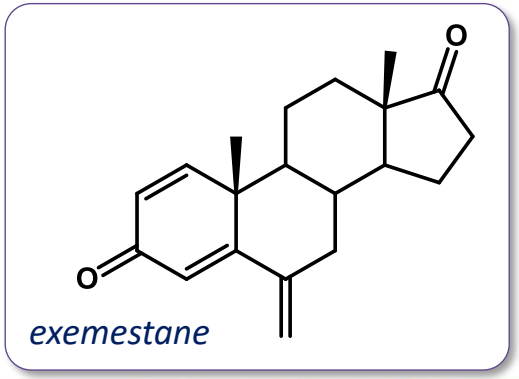
- The first line of treatment in estrogen-dependent BC is the use of antiestrogens and aromatase inhibitors (AIs).
- AIs bind to the substrate-binding site of the aromatase.
- Based on their structure, AIs can be divided in:

Steroidal AIs

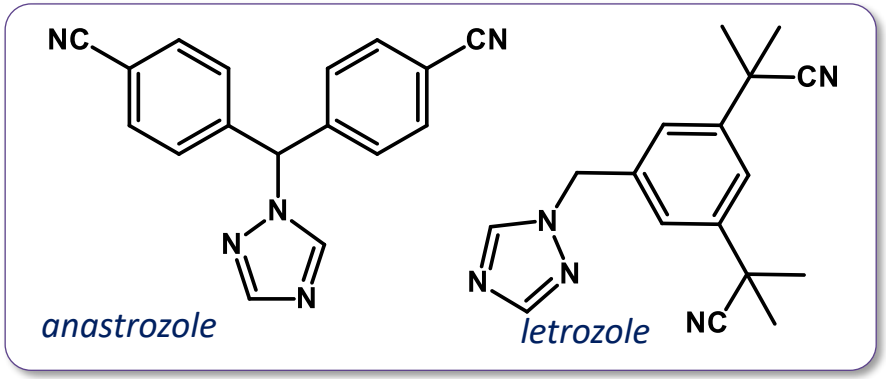
strictly related to androstenedione, bind irreversibly to the active site (irreversible inhibition)

Non-steroidal AIs

coordinate to the heme iron of the enzyme in a reversible manner (reversible inhibition)



3rd generation



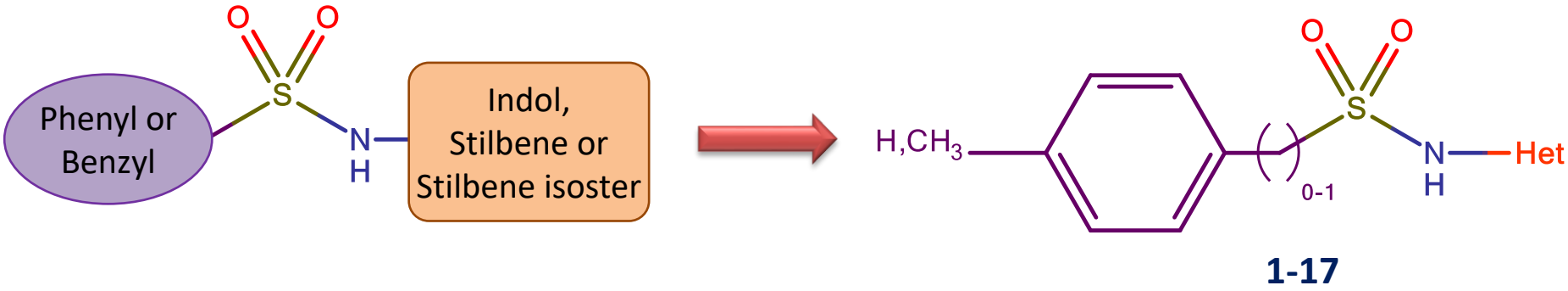
Introduction: Aromatase inhibitors

- 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.

The search of a more active aromatase inhibitor with less side effects is still open.

Aim of the work

- Design and synthesis of a new sulfonamide library
- Biological assays in terms of the evaluation of the enzymatic IC_{50}, cell viability and cellular IC_{50} in MCF7 breast cancer cell lines
- Understand the binding mode of the most active compounds by docking study



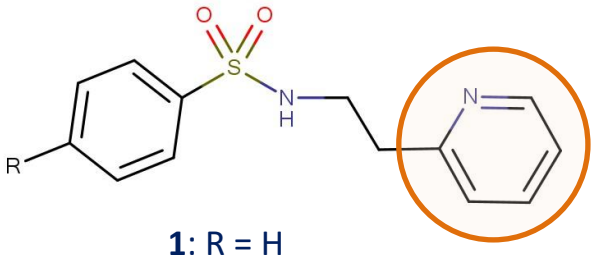
1-17

Fantacuzzi M., Eur. J. Med. Chem. 2020, 185, 111815

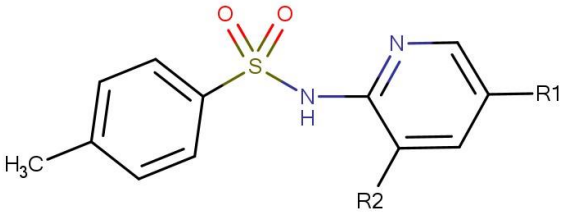
Fantacuzzi M., Pharmaceuticals 2021, 14, 984

Giampietro L., Eur. J. Med. Chem. 2021, 224, 113737

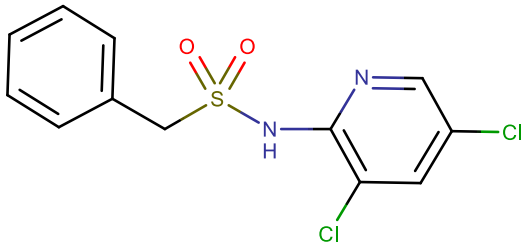
Structure of compounds



1: R = H
2: R = CH_3



3: R1 = R2 = H
4: R1 = Cl, R2 = H
5: R1 = Cl, R2 = Cl



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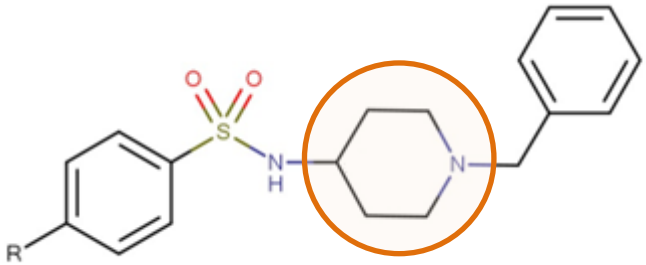
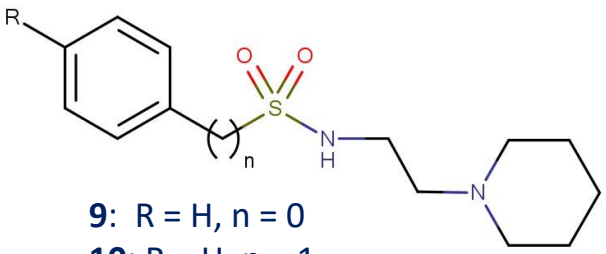
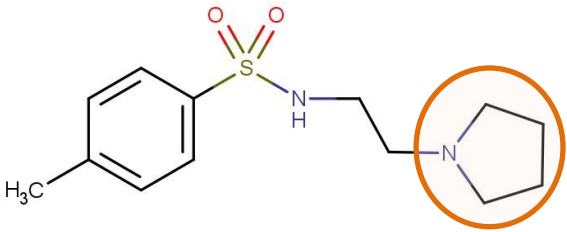


8: R = CH_3



9: R = H, n = 0
10: R = H, n = 1
11: R = CH_3, n = 1



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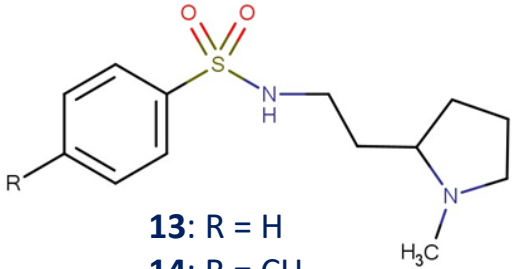
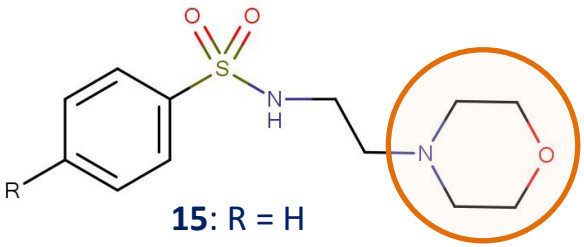
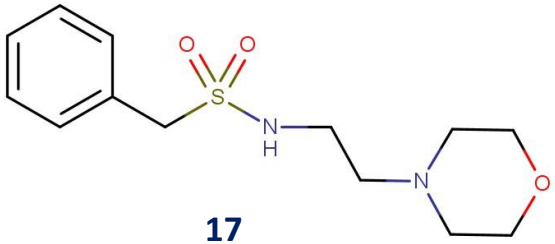


13: R = H
14: R = CH_3



15: R = H
16: R = CH_3



17

Synthesis

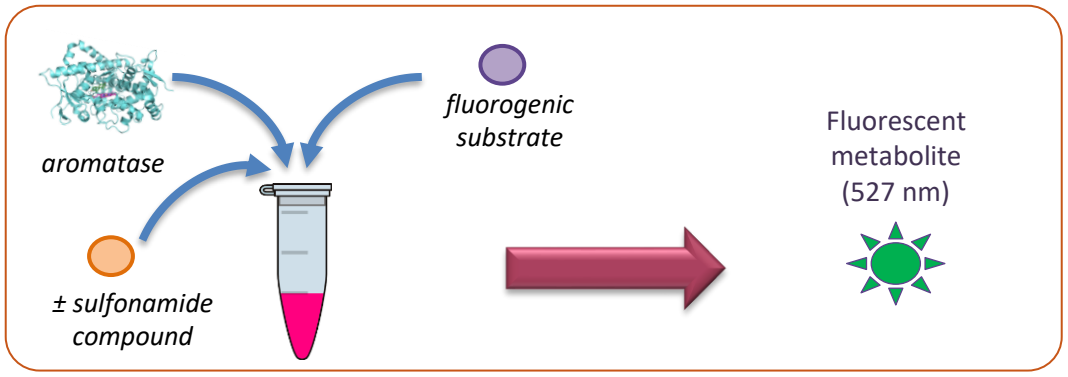
1.0 eq *1.2 eq* **1-17**

Reagents and conditions: 3 eq NEt_3, dry CH_2Cl_2, N_2, 0°C for 2h, r.t. 18h-22h.

- The purification by Liquid Chromatography gave the purified compounds **1-17**.
- Melting points were determined.
- ^{1}H and ^{13}C Nuclear Magnetic Resonance spectra were monitored.
- Elemental analyses were carried out.

Biological assay

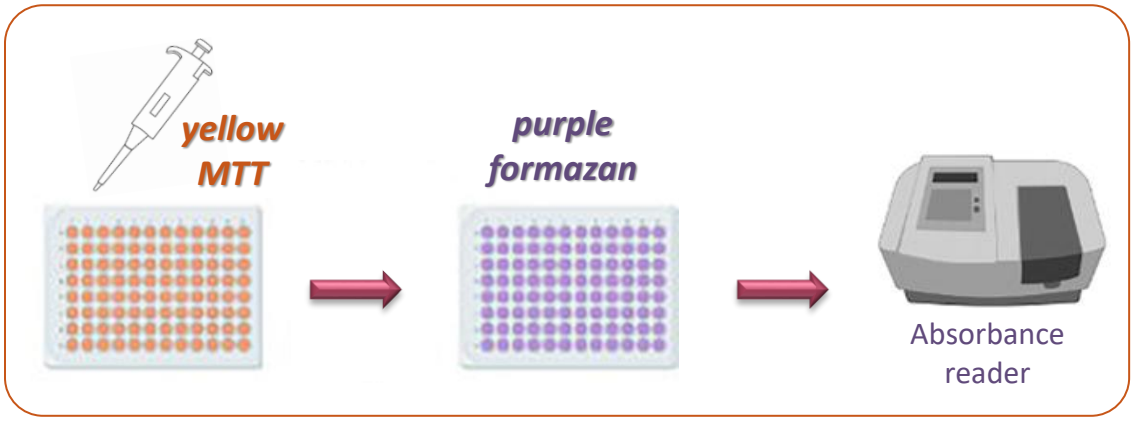
- The *in vitro* anti-aromatase activity was valued using a commercial fluorimetric assay kit using letrozole (LTR) as reference drug.
- Compounds **1-17** were tested at 7 different concentrations (0-100 µM) for IC_{50} calculation.
- The most active compounds (**1, 3, 9-10, 13-14**) showed IC_{50} in the range 32–60 nM.
- Experiments were performed in quadruplicate.



Cmp	IC_{50} (µM)
1	0.060 ± 0.002
2	0.248 ± 0.010
3	0.035 ± 0.001
4	>100
5	>100
6	>100
7	>1000
8	>1000
9	0.032 ± 0.001
10	0.052 ± 0.002
11	>100
12	>1000
13	0.046 ± 0.002
14	0.051 ± 0.001
15	0.337 ± 0.012
16	0.160 ± 0.006
17	0.206 ± 0.009
LTR	0.026 ± 0.001

Biological assay

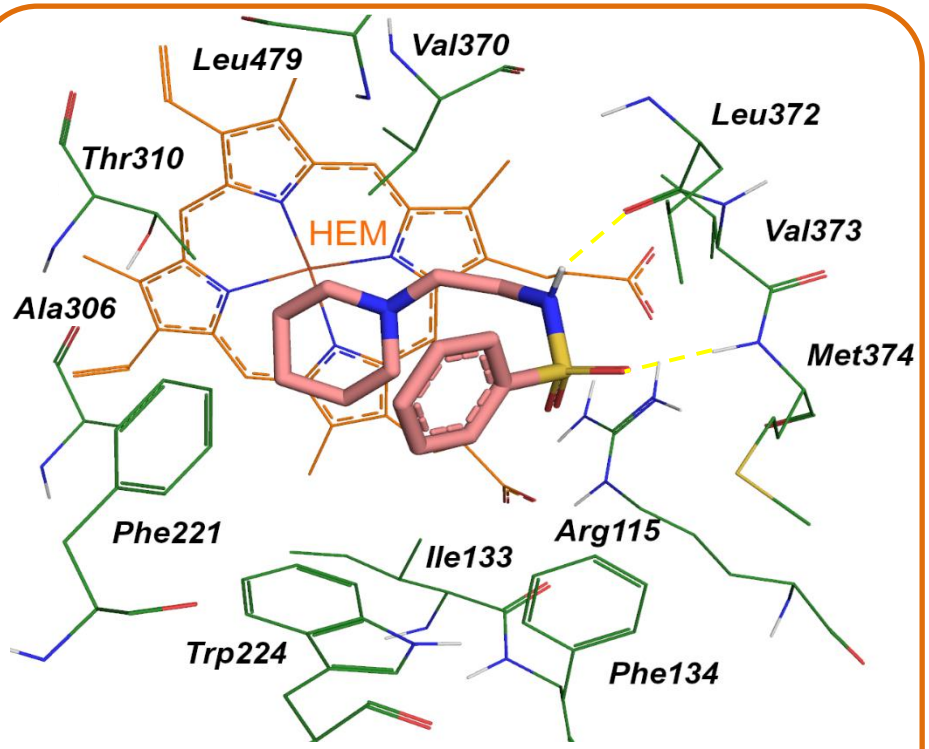
- The cellular viability was assessed by MTT assay in the breast cancer MCF7 cell line using doxorubicin (DOX) as reference drug.
- Compounds were tested at 7 different concentrations (0-100 µM) for cellular IC_{50} calculation.
- The most active compounds (**1, 3, 9-10, 13-14**) in enzymatic assay showed good cellular IC_{50}.
- Experiments were repeated in quadruplicate.



Cmp	MCF7 IC_{50} (µM)
1	7.511 ± 0.296
2	15.410 ± 0.356
3	2.679 ± 0.104
4	> 100
5	> 100
6	25.311 ± 0.945
7	28.469 ± 0.514
8	> 100
9	3.288 ± 0.119
10	6.201 ± 0.258
11	43.617 ± 1.863
12	32.062 ± 1.166
13	8.062 ± 0.319
14	10.505 ± 0.477
15	12.386 ± 0.417
16	19.579 ± 0.678
17	14.613 ± 0.522
DOX	1.940 ± 0.084

Docking study

- The best active compounds bind the aromatase active site via most of the binding residues of androstenedione that include Phe134, Trp224, Val370, Val373 and Met374
- The cofactor heme group (HEM) plays an essential role in binding interacting with the phenyl or the heterocycle of new compounds
- In addition, as for the most active compound **9**, two H-bonds (*yellow dashed line*) can be established between the sulfonamide group and the two residues Leu372 and Met374.



*Compound **9** in pink, HEM in orange, and key residues of aromatase (PDB:3EQM) in green.*

Conclusions

- A library of 17 new sulfonamide compounds was synthesized by a one-step chemical process starting from commercially available products.
- The capability to inhibit aromatase enzyme was evidenced for six compounds (**1**, **3**, **9**, **10**, **13** and **14**) showing IC_{50} values in the range of nanomolar (32–60 nM).
- The cell viability and IC_{50} on MCF7 demonstrated a good activity to kill breast cancer cell
- The docking study revealed that the best active compounds interact with the key residues in the active site of the enzyme and the sulfonamide group can establish two H-bonds with the amino and the carboxy groups of Leu372 and Met374 backbone.

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