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

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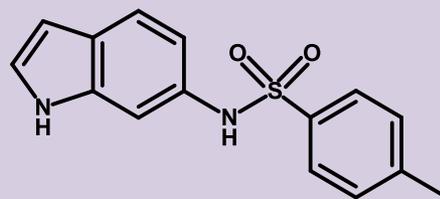
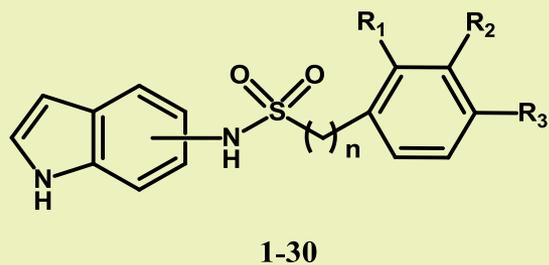
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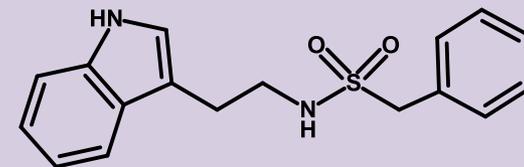


Synthesis, biological evaluation, and docking study of indole aryl sulfonamides as aromatase inhibitors



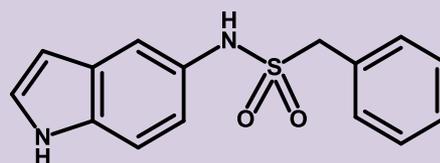
3

IC_{50} : 0.49 μ M



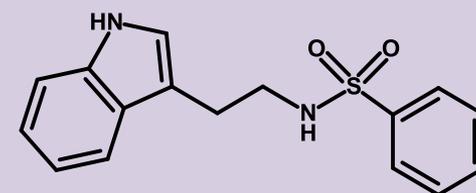
22

IC_{50} : 0.75 μ M



7

IC_{50} : 0.19 μ M



23

IC_{50} : 0.20 μ M



Abstract

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.

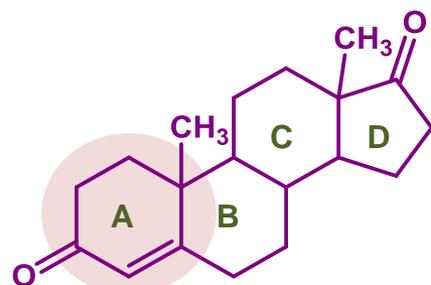
Keywords: aromatase, breast cancer, aromatase inhibitors, sulfonamide, indole, docking, QSAR

[1] J. Chan, K. Petrossian, S. Chen, *J. Steroid. Biochem. Mol. Biol.* 161 (2016) 73–83; [2] G. Waks, E.P. Winer, *JAMA* 321 (2019) 288-300; [3] A. Sychev, G.M. Ashraf, et al., *Drug Des. Devel. Ther.* 12 (2018) 1147–1156; [4] M. Di Matteo, A. Ammazalorso, et al., *Bioorg. Med. Chem. Letters* 26 (2016) 3192–3194.



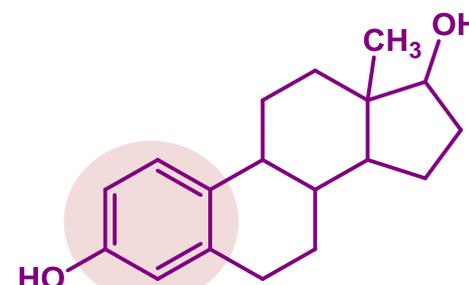
Introduction

- 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.

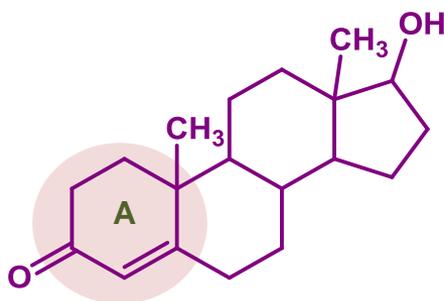


androstenedione

aromatase

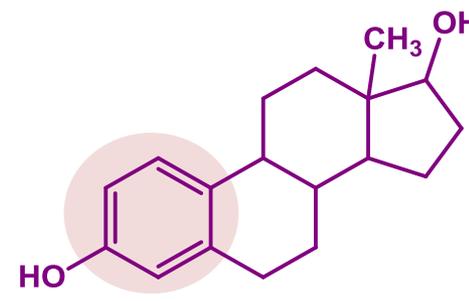


estrone



testosterone

aromatase

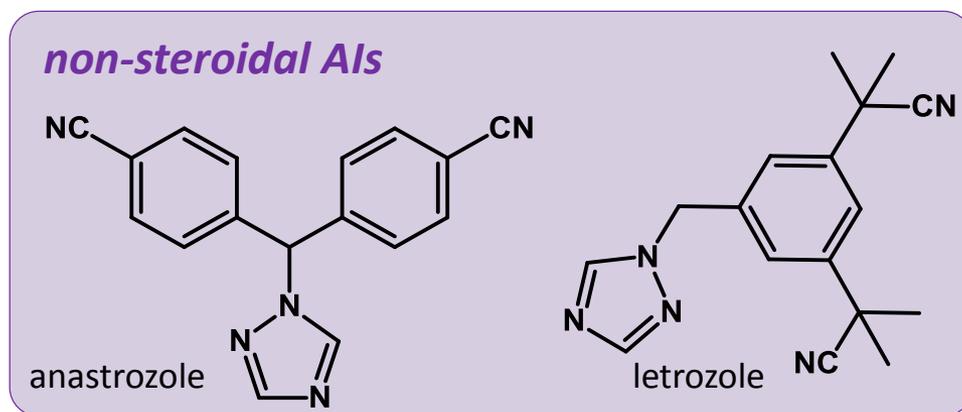
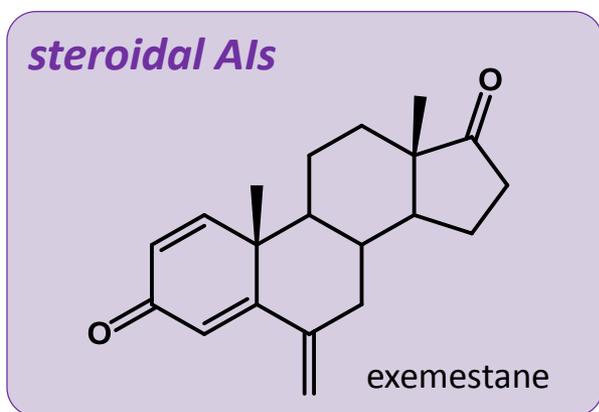


17β-estradiol



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).

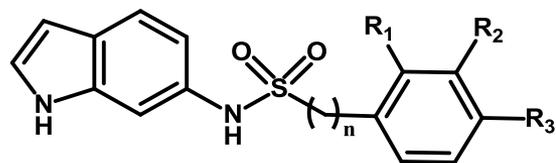


- The third generation of AIs is potent and specific, with strong effect and well tolerated.
- Side effects include bone loss, joint pain, cardiac events.
- Acquired resistance could be developed during the five years therapy.

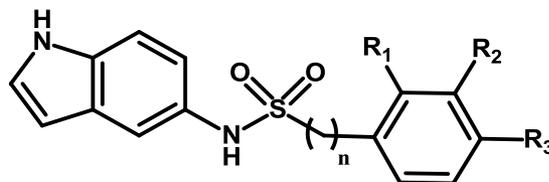


Aim of the work

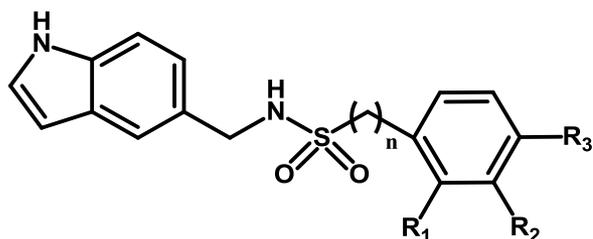
- The discovery of non-steroidal sulfonamide-containing AIs is the aim of this work.
- A library of 30 aryl sulfonamide (**1-30**) is synthesized, the percentage of aromatase inhibition and the IC_{50} are valued, cell viability and cytotoxicity are tested, a docking study and QSAR are realized.



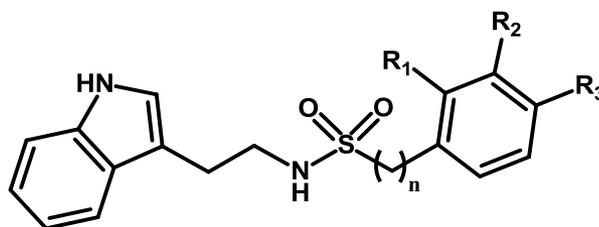
1-6



7-12



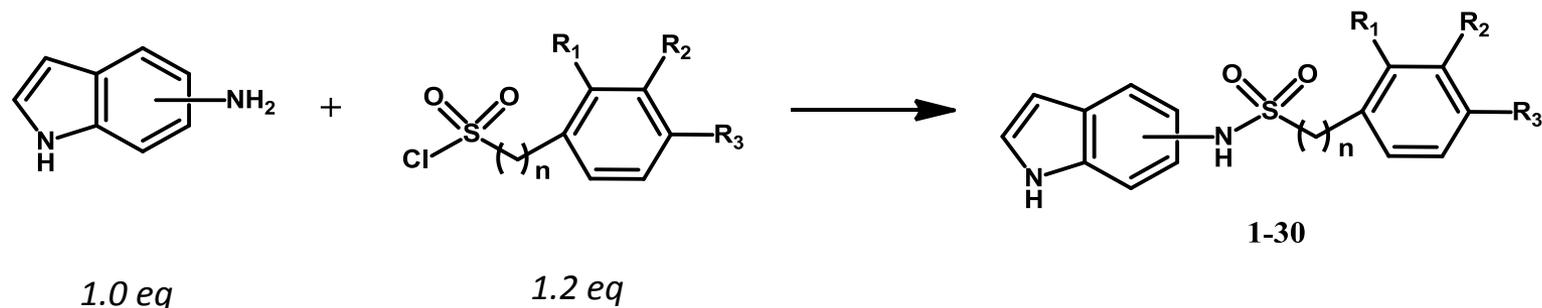
13-21



22-30

Cmp	n	R ₁	R ₂	R ₃
1, 7, 13, 22	1	H	H	H
2, 8, 14, 23	0	H	H	H
3, 9, 15, 24	0	H	H	CH ₃
4, 10, 16, 25	0	H	NO ₂	H
5, 11, 17, 26	0	H	NO ₂	CH ₃
6, 12, 18, 27	0	Cl	H	CN
19, 28	0	H	H	CN
20, 29	0	OCH ₃	H	OCH ₃
21, 30	0			





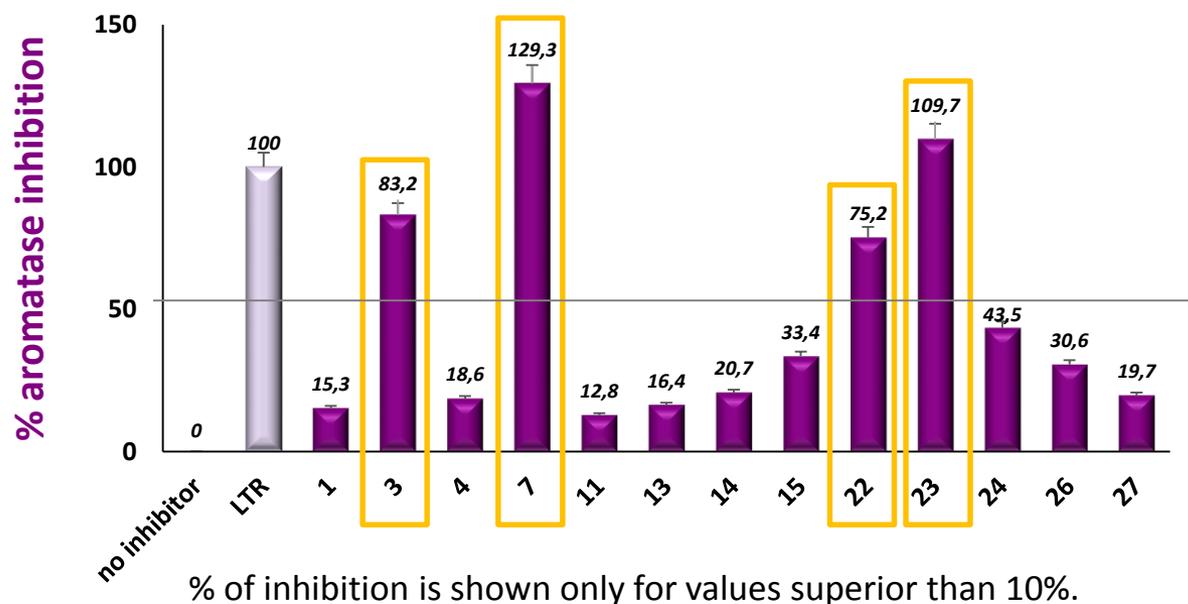
Reagents and conditions: 3 eq NEt_3 , dry CH_2Cl_2 , N_2 , 0°C for 2h, r.t. 18h-22h.

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

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- The *in vitro* anti-aromatase activity was valued using a commercial fluorimetric assay kit using letrozole (LTR, IC₅₀ = 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 IC₅₀ calculation.
- Experiments were repeated in triplicate.



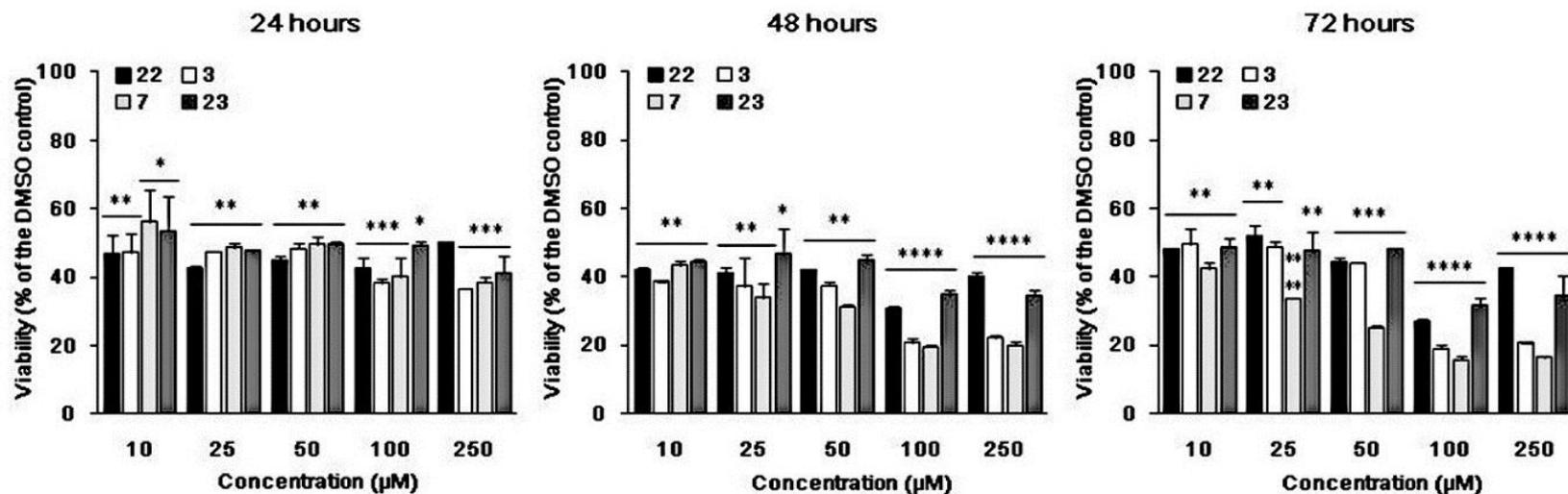
Cmp	IC ₅₀ (μM)
3	0.49 ± 0.03
7	0.16 ± 0.01
22	0.75 ± 0.05
23	0.20 ± 0.01

^aThe value represents mean of triplicate determinations ±RSD

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- 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**.

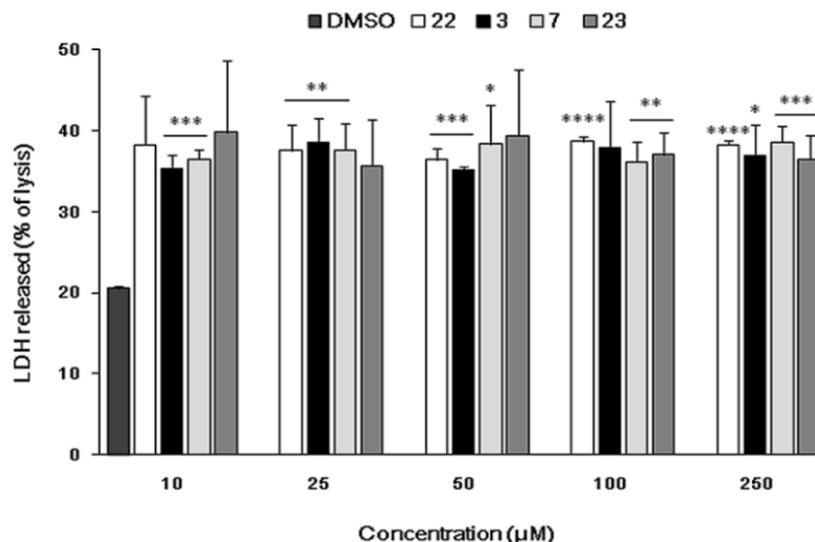


- A time and dose-dependent decrease with loading concentrations of **3**, **7**, **22-23** were revealed.
- Cell metabolic activity is assessed at around 40-45% after the exposure to 10 μM at 48 h.
- Compounds **3** and **7** reach values of metabolic activity around 20% at higher doses.

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- 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 μ M for 24 h.



- There is a secretion of the enzyme assessed 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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- 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).

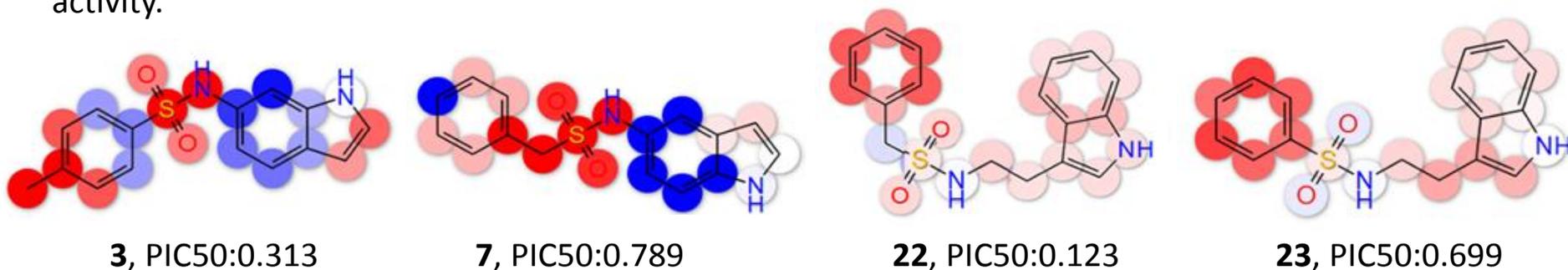
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- The atomic contribution maps of the four compounds showed considerable aromatase inhibitory activity.



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_{50} values in the range of sub-micromolar (0.16-0.75 μ M).
- 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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