6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
sciforum.net/conference/ECMC2020
sponsored by pharmaceuticals

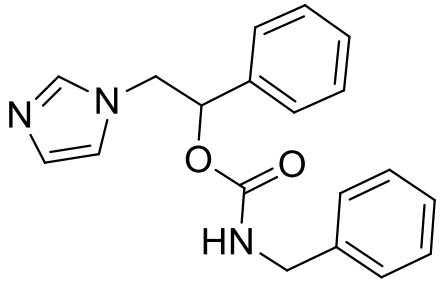
Imidazole and Triazole-based Carbamates as Novel Aromatase Inhibitors: Synthesis and *in vitro* Evaluation on MCF7 Breast Cancer Cells

Alessandra Ammazzalorso

Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, Via dei Vestini, 66100 Chieti, ITALY

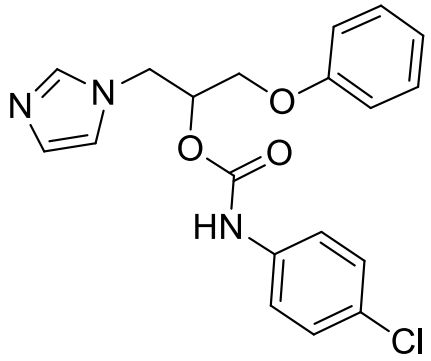
alessandra.ammazzalorso@unich.it

Imidazole and Triazole-based Carbamates as Novel Aromatase Inhibitors: Synthesis and *in vitro* Evaluation on MCF7 Breast Cancer Cells



13a

IC_{50} (aromatase) 0.82 μM
IC_{50} (MCF7) 6.58 μM



15c

IC_{50} (aromatase) 0.86 μM
IC_{50} (MCF7) 7.16 μM

Abstract

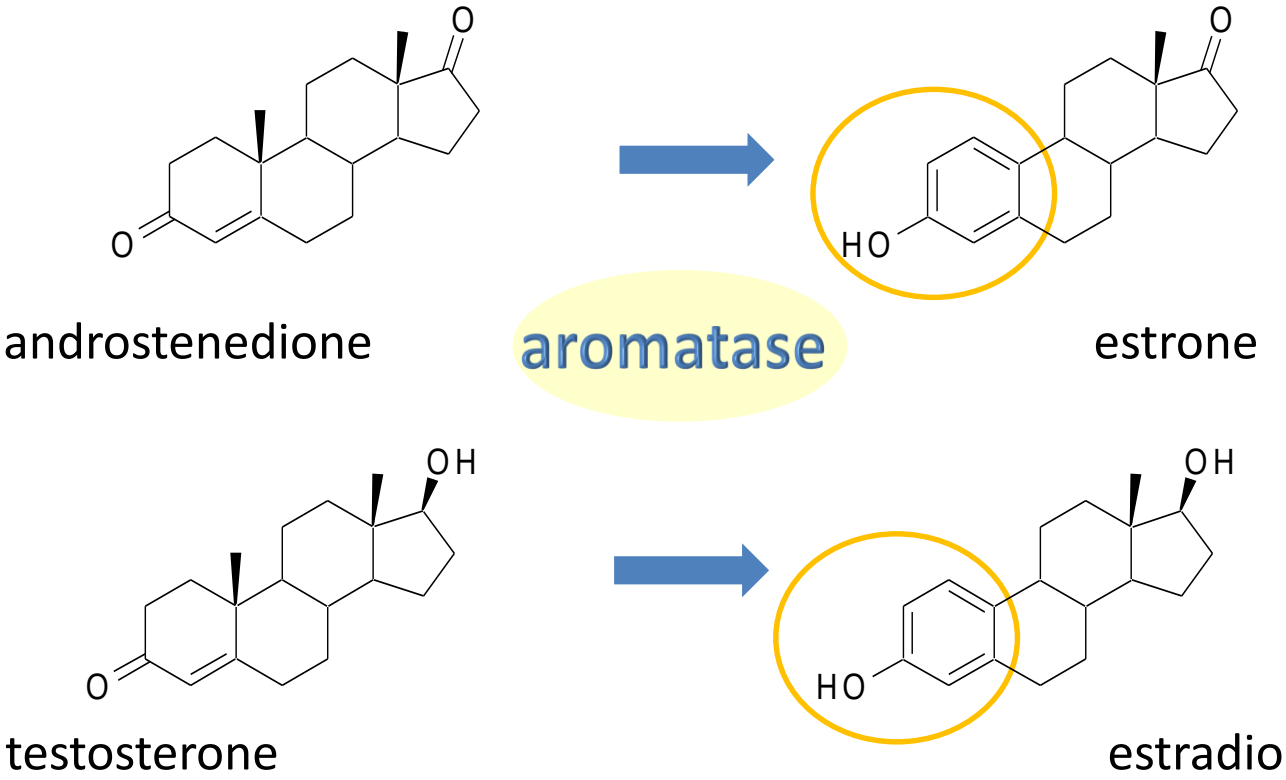
Aromatase (CYP19), a member of cytochrome P450 enzymes, is a monooxygenase responsible for the conversion of androgens (androstenedione and testosterone) to estrogens (estradiol and estrone). Estrogens play a central role in the development and progression of breast cancer: aromatase inhibitors and antiestrogens represent first line treatments in post-menopausal estrogen-dependent breast cancer. Extensive research over the last decades led to the identification of several aromatase inhibitors, classified as steroidal and non-steroidal, acting by a competitive or not competitive mechanism of action.

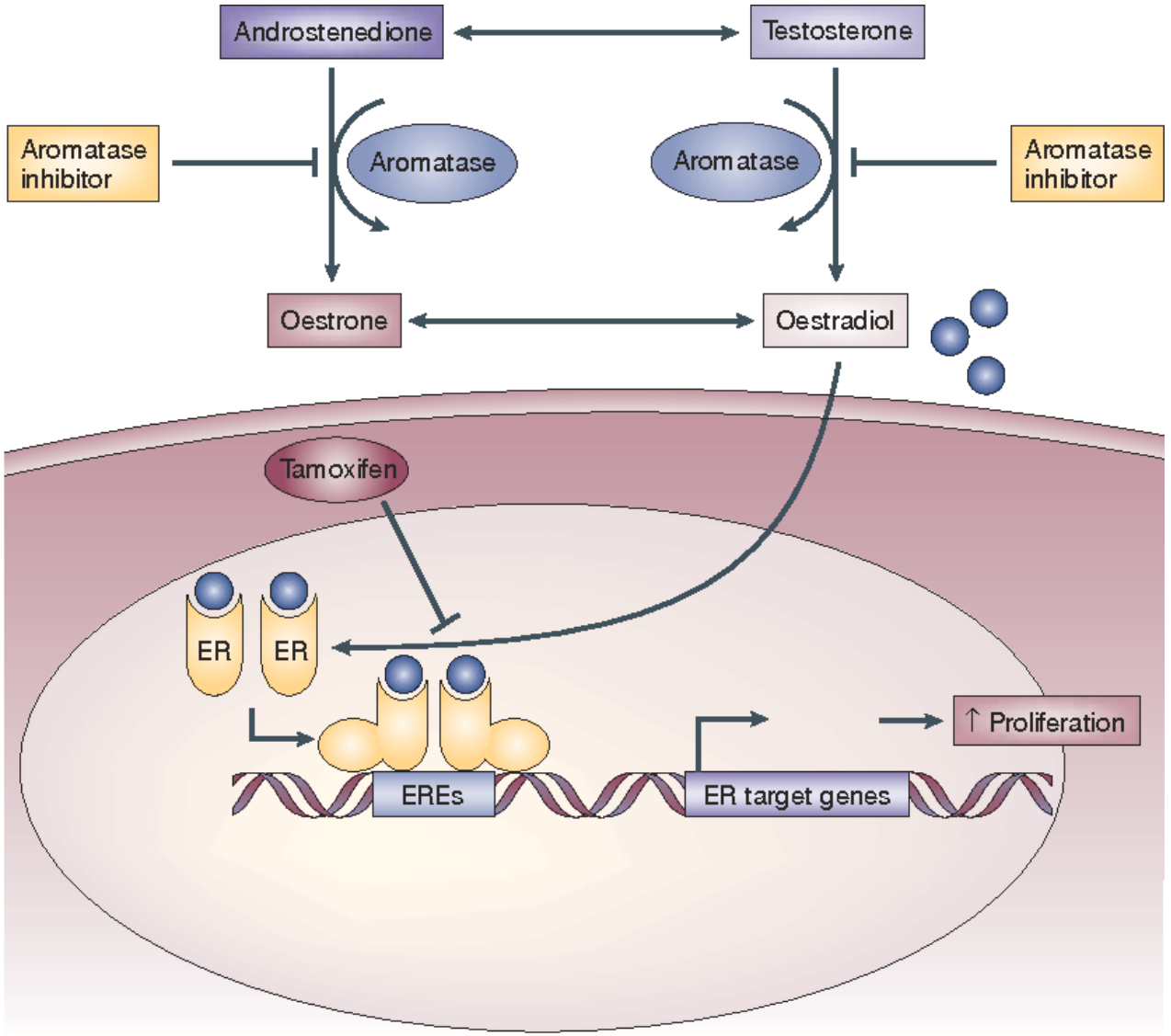
Our research group is involved in the discovery of novel non-steroidal aromatase inhibitors, based on azole scaffold. In the present study, novel compounds were designed by modification of BAS02077837, an imidazole-based aromatase inhibitor. Synthesized molecules were evaluated against aromatase, in a fluorimetric *in vitro* assay, and compared to letrozole. The most active derivatives were then submitted to cell viability and cytotoxicity evaluation on MCF7 breast cancer cells. Docking studies were also performed on most promising compounds to shed light on their binding mode in human aromatase binding pocket.

Keywords: aromatase inhibitors, breast cancer, carbamates, imidazole, triazole.

Introduction

Aromatase, a monooxygenase coded by the gene CYP19, is responsible for the conversion of androgens to estrogens by demethylation and aromatization of the steroidal A-ring.
Androstenedione is the endogenous substrate of aromatase.

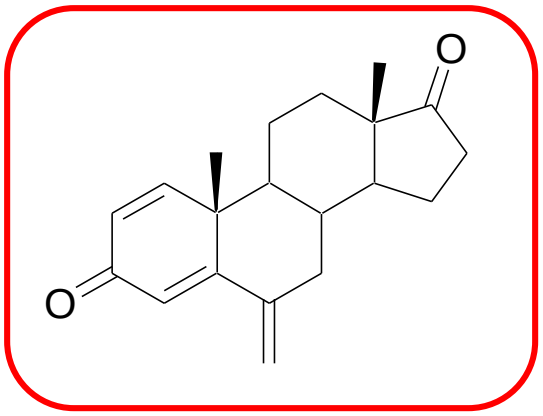


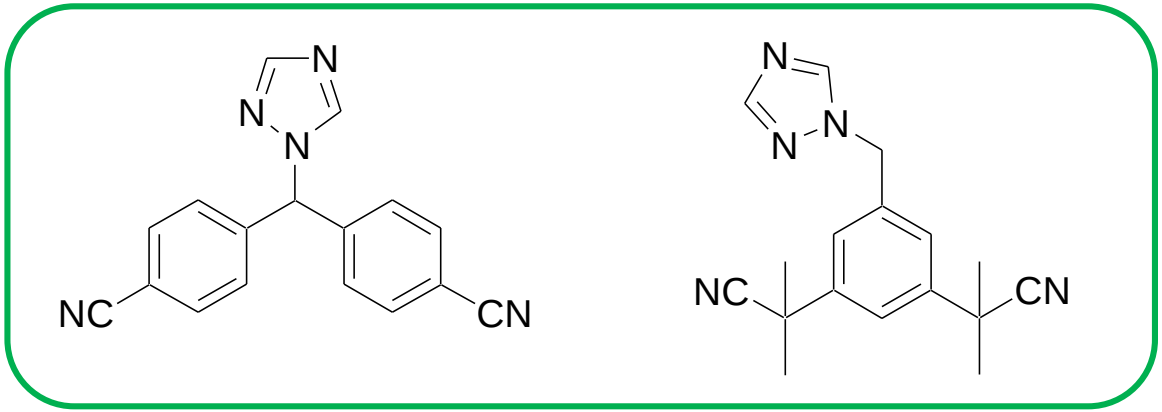


Nat Rev Cancer. 2003 Nov;3(11):821-31

Aromatase Inhibitors

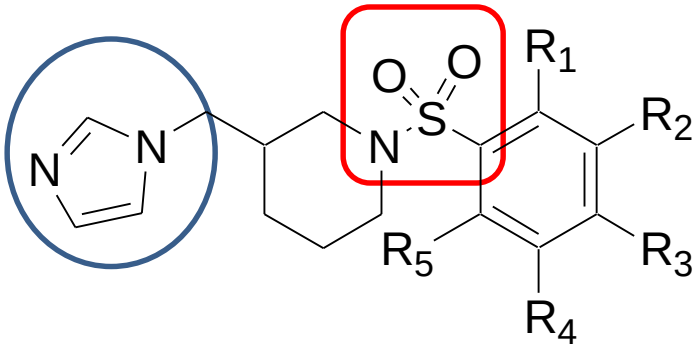
Steroidal		Non Steroidal
	First generation	Aminoglutetimide
Formestane	Second generation	Fadrazole
Exemestane	Third generation	**Letrozole, Anastrozole** Vorozole





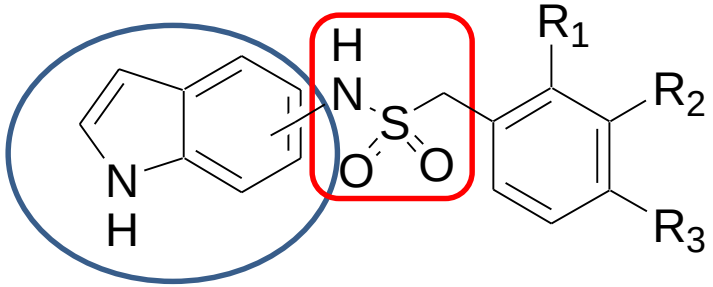
Aromatase Inhibitors

Two families of azole-based sulfonamides were developed by our research group as novel aromatase inhibitors.



In vitro enzymatic activity
IC_{50} 6-339 nM

Bioorg Med Chem Lett. 2016, 26, 3192-3194

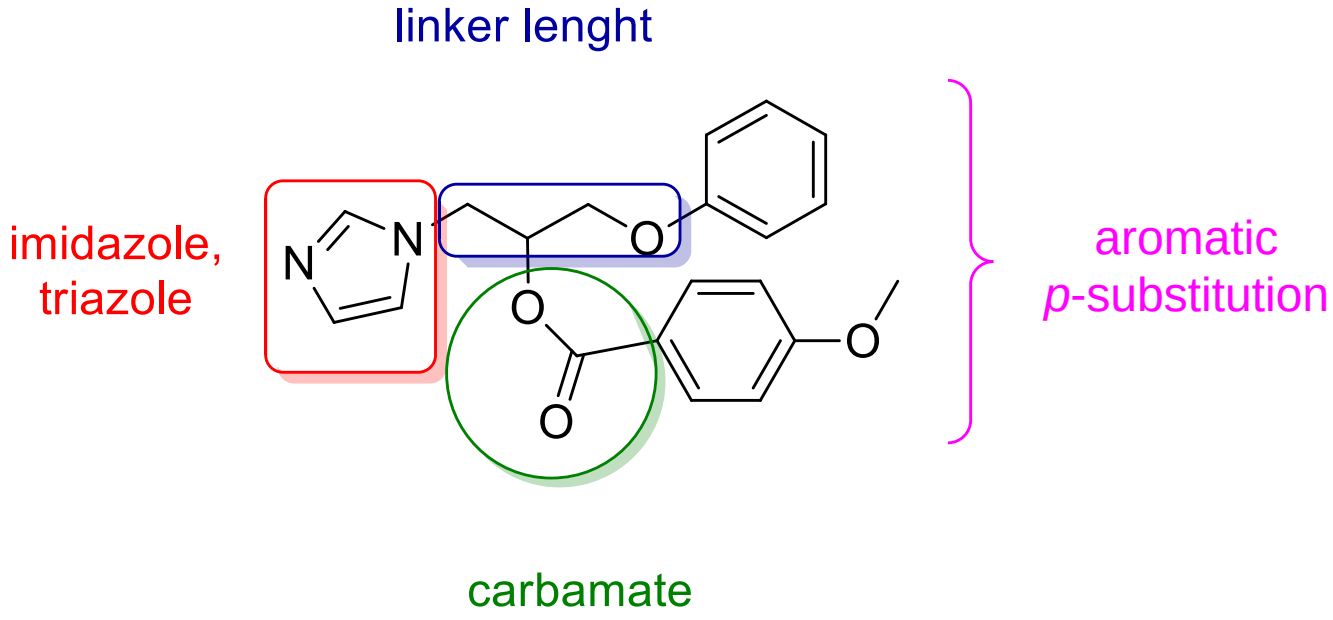


In vitro enzymatic activity
IC_{50} 0.16-0.75 μM

Eur J Med Chem. 2020, 185, 111815

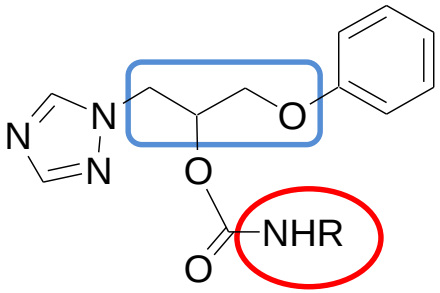
Aim of the Work

To identify novel azole-based aromatase inhibitors, starting from the structure of BAS02077837

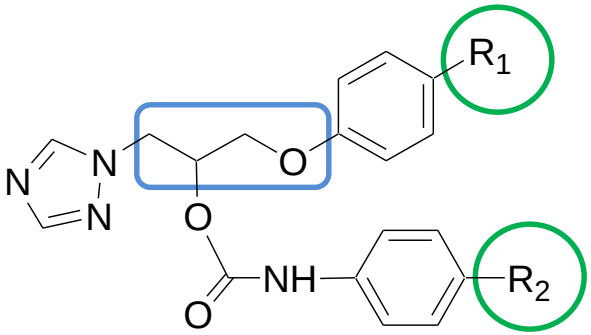


Designed Carbamates

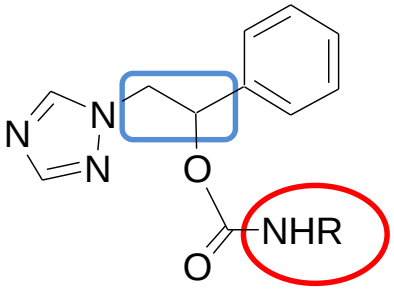
Series I: Triazole Derivatives



R = Bn, Ph, *p*Cl-Ph, *p*OCH_3-Ph, *o,p*OCH_3-Ph



R_1 = H, Cl, OCH_3
R_2 = H, Cl, OCH_3



R = Bn, Ph, *p*Cl-Ph

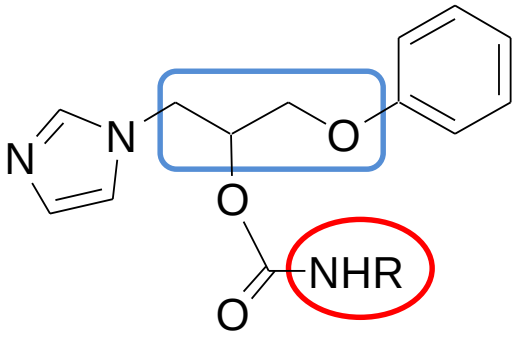
Lenght of the linker

para substitution on aromatic rings

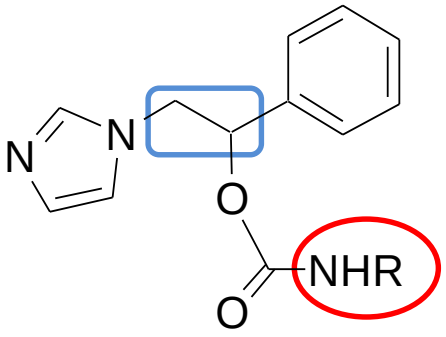
substitution on carbamic function

Designed Carbamates

Series II: Imidazole Derivatives



R = Bn, Ph, *p*Cl-Ph, *p*OCH_3-Ph, *o*,*p*OCH_3-Ph

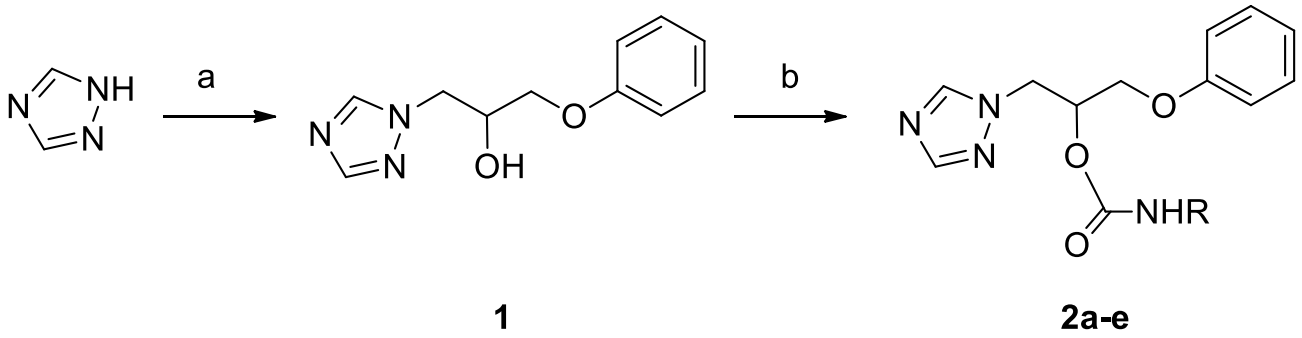


R = Bn, Ph, *p*Cl-Ph

Lenght of the linker

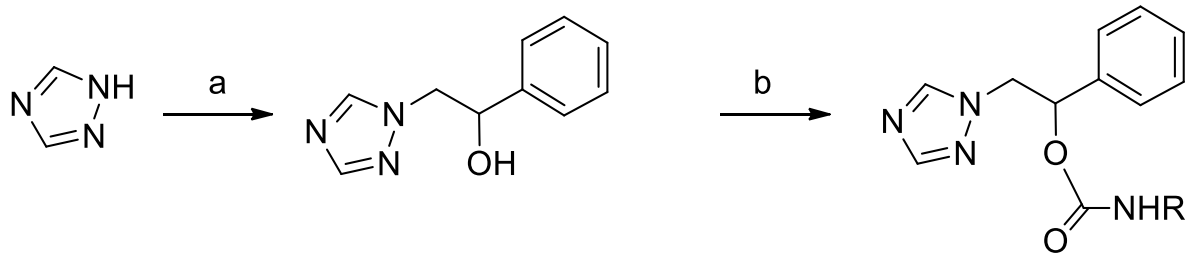
substitution on carbamic function

Synthesis of Triazole Carbamates 2a-e and 11a-c



1 **2a-e**

a R = Bn
b R = Ph
c R = *p*Cl-Ph
d R = *p*OCH_3-Ph
e R = *o,p*OCH_3-Ph



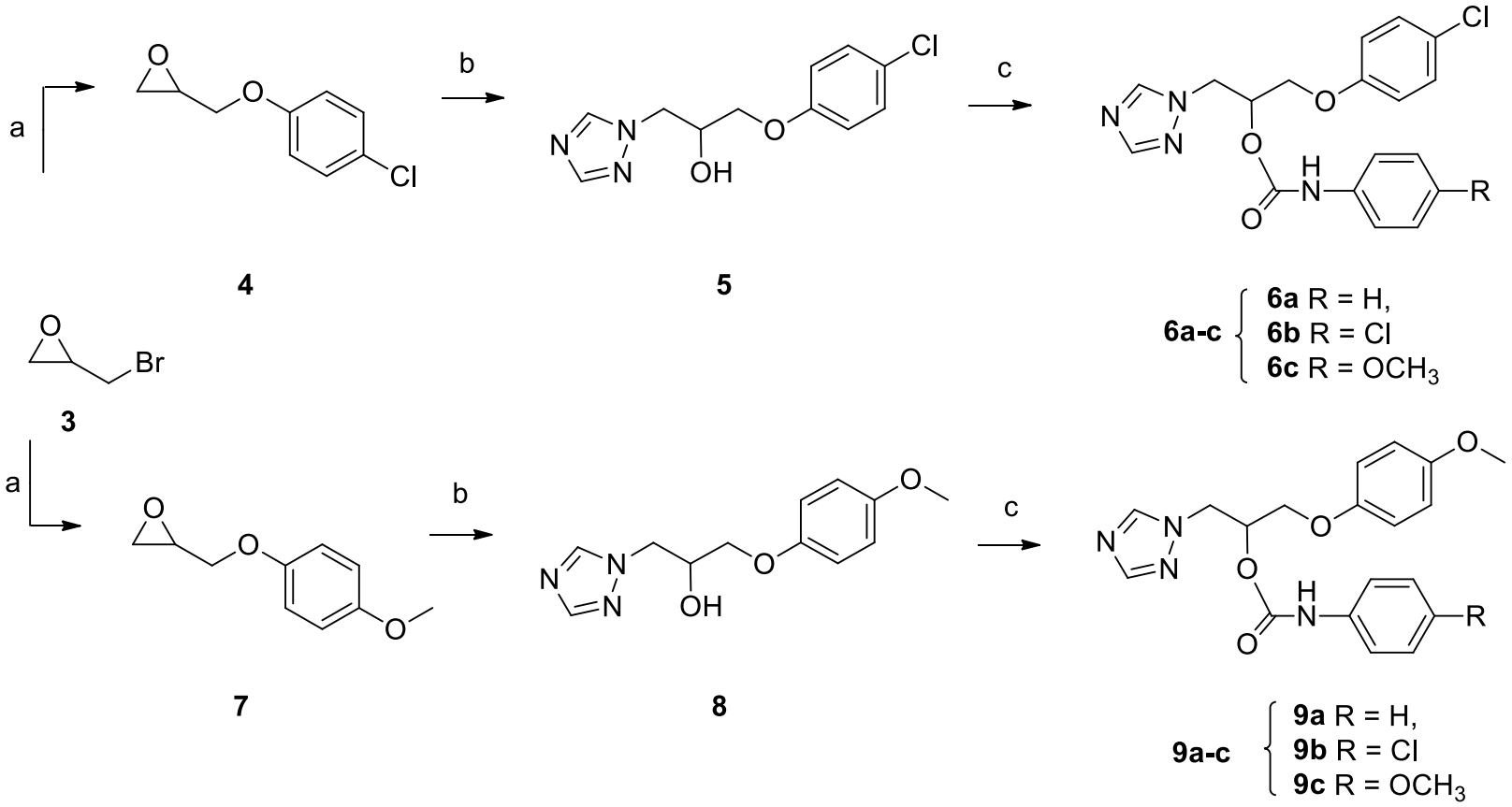
10 **11a-c**

a R = Bn
b R = Ph
c R = pCl-Ph

Reagents and conditions: a) 1,2-epoxy-3-phenoxypropane (for **1**), styrene oxide (for **10**) THF, 60°C, 24h; b) sodium hydride, R-NCO, dry ACN, N_2, r.t., 24h.

Ammazzalorso A. et al, under submission

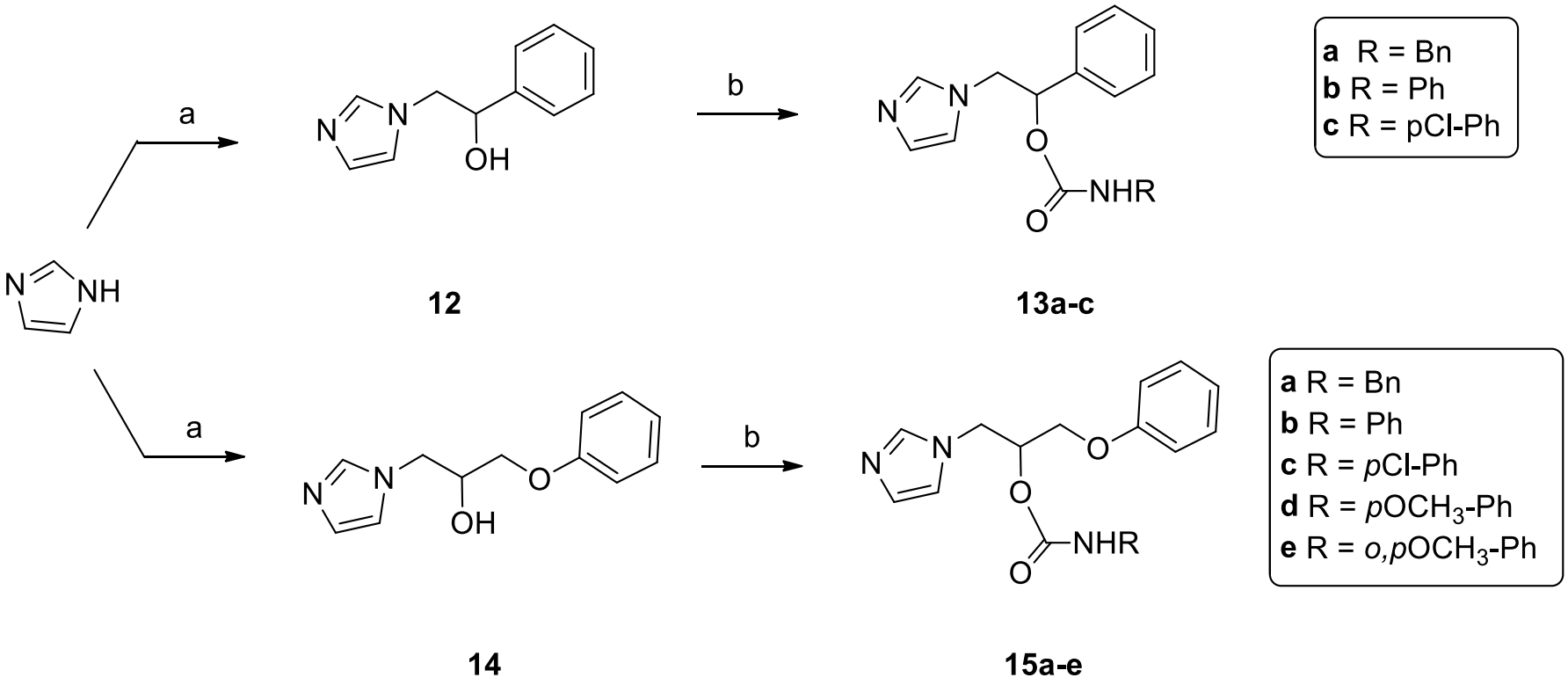
Synthesis of Triazole Carbamates 6a-c and 9a-c



Reagents and conditions: a) *p*-chlorophenol (for **4**) or *p*-methoxyphenol (for **7**), potassium carbonate, refluxing ACN, 7h; b) triazole, THF, 60°C, 24h; c) sodium hydride, R-NCO, dry ACN, N_2, r.t., 24h.

Ammazzalorso A. et al, under submission

Synthesis of Imidazole Carbamates 13a-c and 15a-e



Reagents and conditions: a) styrene oxide (for **12**) or 1,2-epoxy-3-phenoxypropane (for **14**), THF, 60°C, 24h; b) sodium hydride, R-NCO, dry ACN, N_2, r.t., 24h.

Ammazzalorso A. et al, under submission

In vitro Aromatase Inhibition Assay

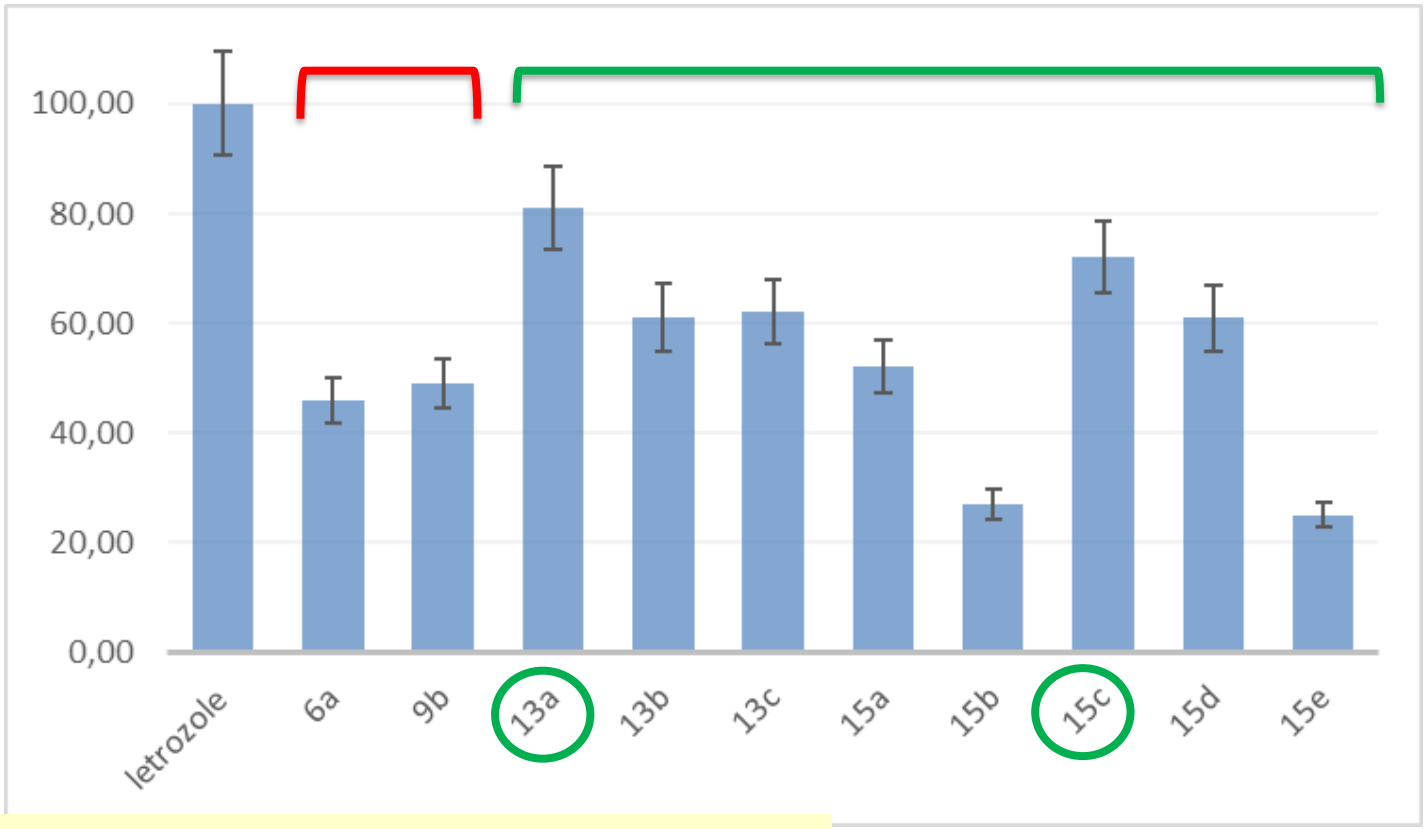
- The human aromatase inhibitory activity of the 22 synthesized compounds was determined by using an ***in vitro*** **fluorescence-based assay** Aromatase (CYP19A) Inhibitor Screening Kit (Fluorimetric), BioVision.

- This assay utilizes a fluorogenic substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 488/527 nm).

- The activity was measured dissolving target compounds in acetonitrile (1 μM), and the results were compared to letrozole at the same concentration, used as reference compound.

In vitro Aromatase Inhibition Assay: Results

Triazole derivatives **Imidazole derivatives**

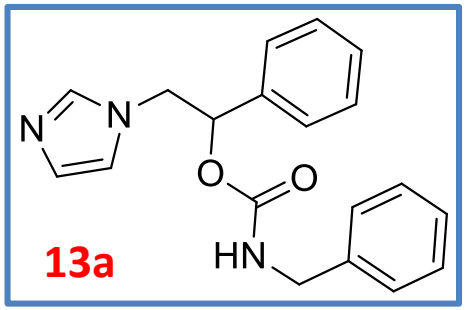


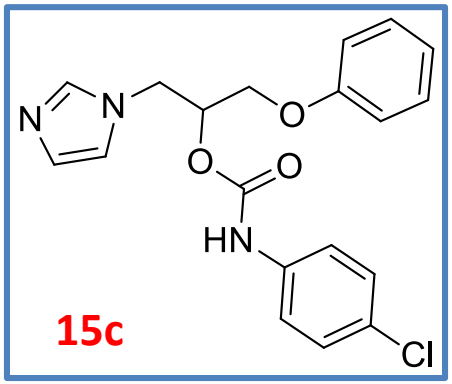
% of inhibition is shown only for values superior than 20%, compared to letrozole taken as 100%

Ammazzalorso A. et al, under submission

In vitro Aromatase Inhibition Assay: Results

Cpd	Aromatase inhibition %[a]	IC_{50} (μM)[b,c]
13a	81	0.82 ± 0.02
15c	72	0.86 ± 0.05



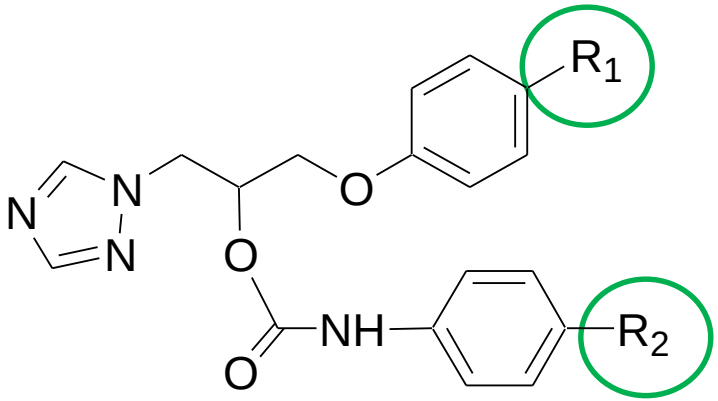


[a] Inhibition percentage was calculated with respect to letrozole (1 μM), taken as 100%; [b]each compound was tested at concentration from 0.01 to 100 μM; [c] the values represent mean of triplicate determinations ± RSD

Ammazzalorso A. et al, under submission

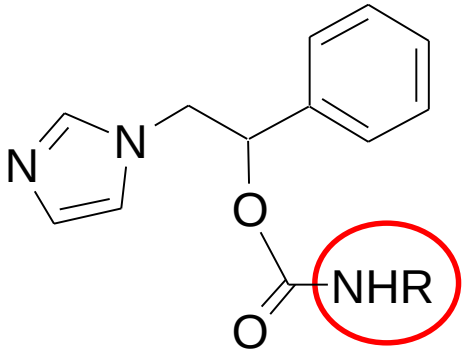
In vitro Aromatase Inhibition assay: Structure-Activity Relationships

Series I: Triazole Derivatives



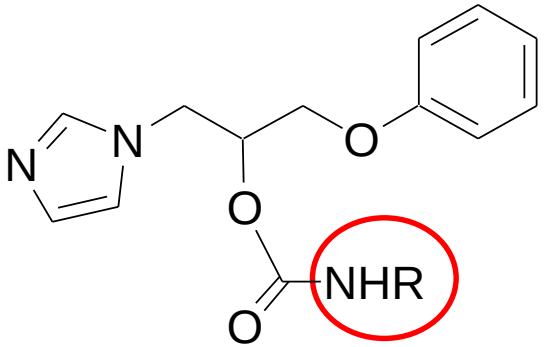
6a R_1 = Cl, R_2 = H
9b R_1 = OCH_3, R_2 = Cl

Series II: Imidazole Derivatives



13

Benzyl >> Phenyl

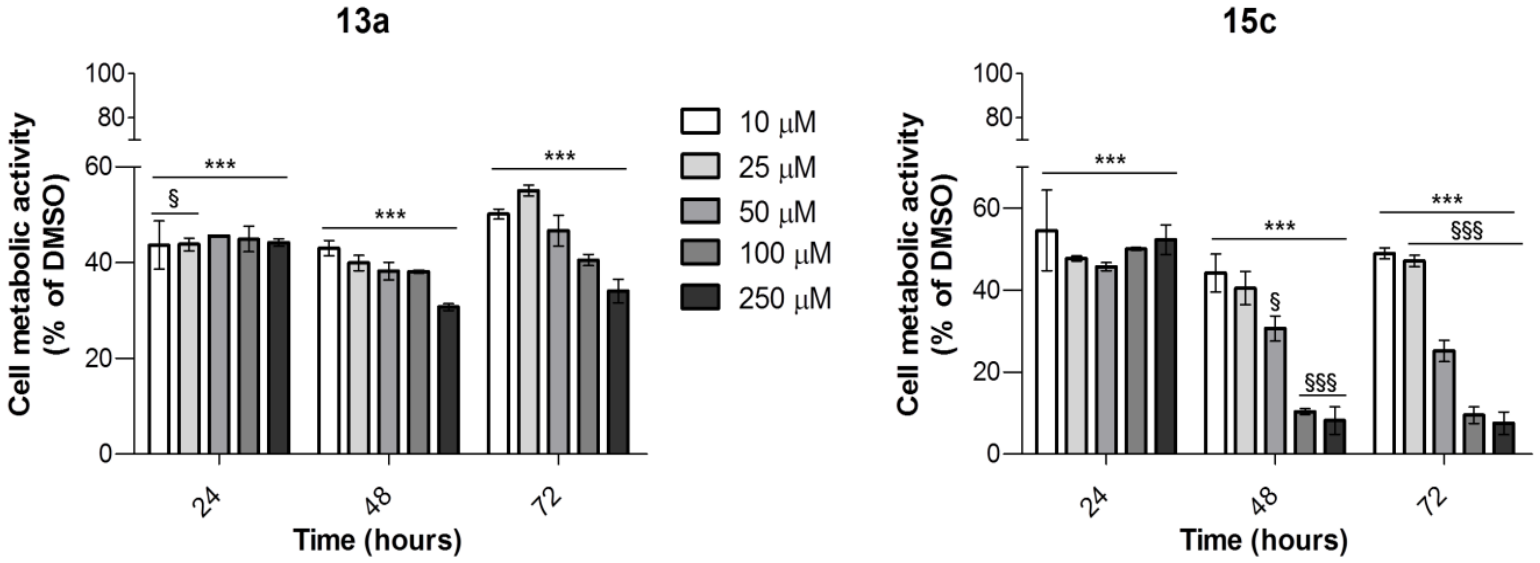


15

p-Cl Ph > *p*-OMe Ph > Bn >> Ph, *o,p*-OMe Ph

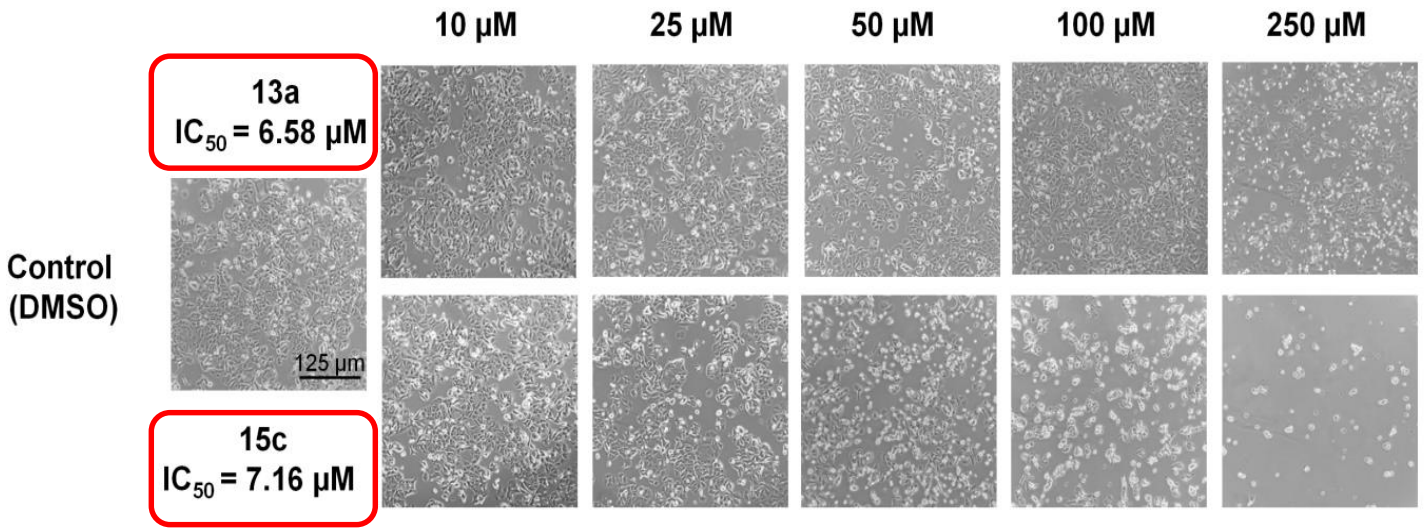
Cell Viability Evaluation on Human MCF7 Cells by MTT Assay

Most active compounds **13a** and **15c** were then submitted to an *in vitro* evaluation on breast cancer cell line MCF7 by MTT assay, cytotoxicity assay (LDH) and cell cycle analysis.



Ammazzalorso A. et al, under submission

Cell Viability Evaluation on Human MCF7 Cells by MTT Assay

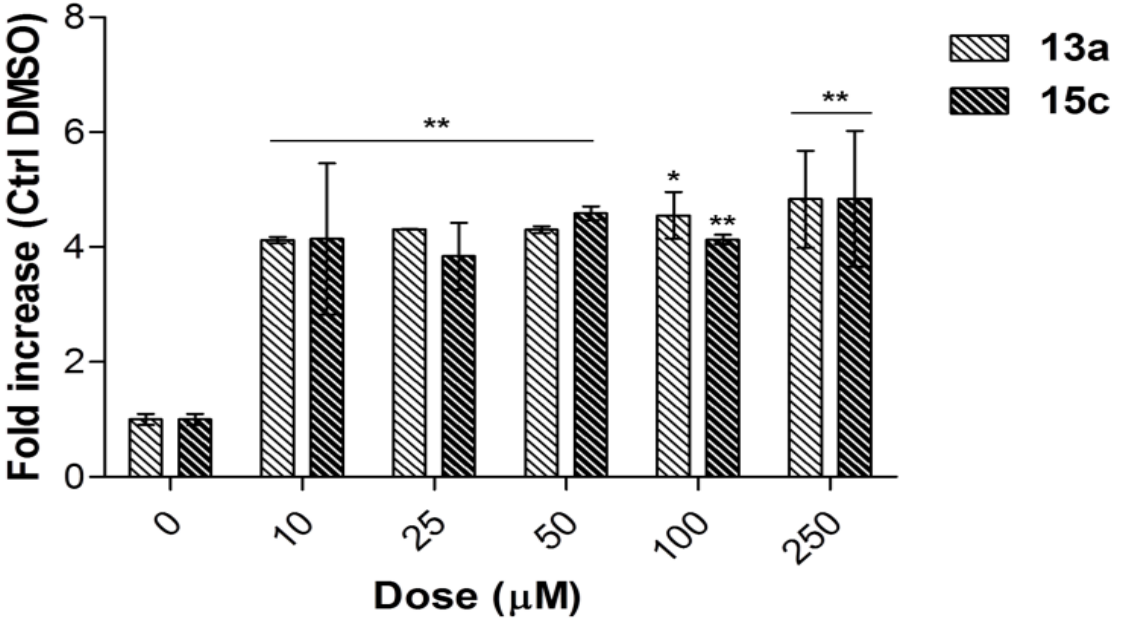


Light microscopy images of MCF7 cells cultivated in 6 well plates (2.5 x10^5/well) in the presence of DMSO (control) and loading concentrations of compounds **13a** and **15c** for 72 hours.

Ammazzalorso A. et al, under submission

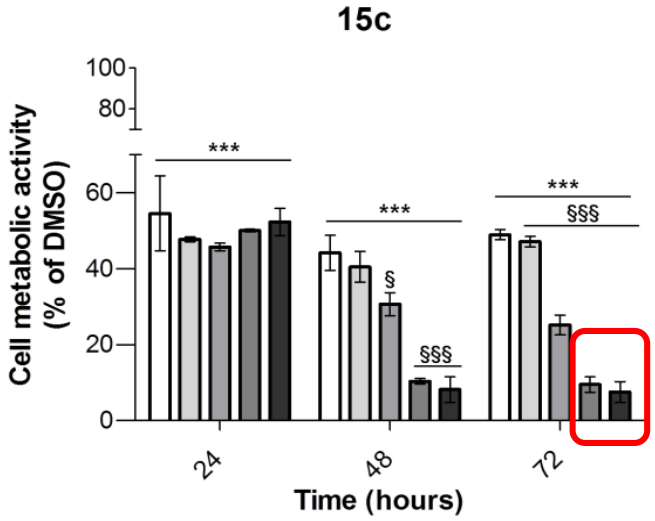
Cytotoxicity Evaluation on MCF7 Cells by LDH Assay

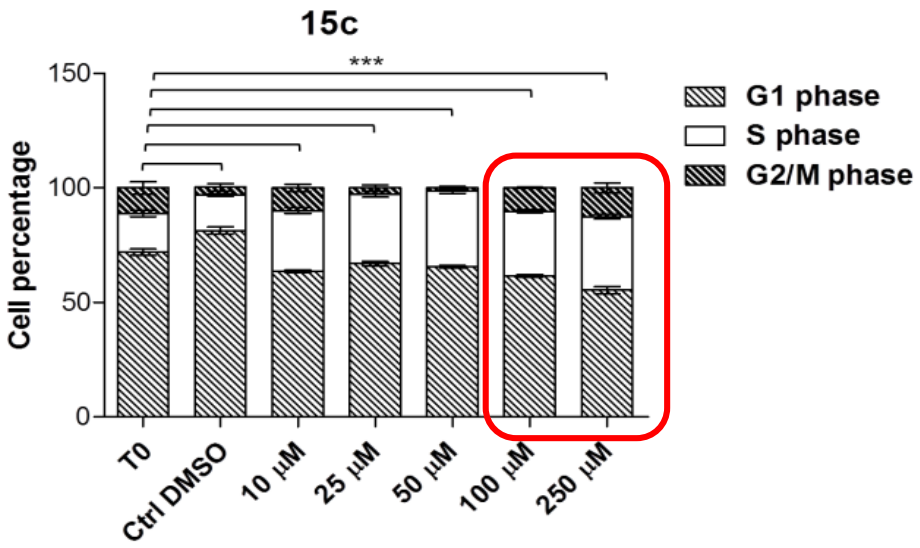
The cytotoxic effect of both compounds was measured by the lactate dehydrogenase assay of MCF7 cells in response to loading concentrations of **13a** and **15c**

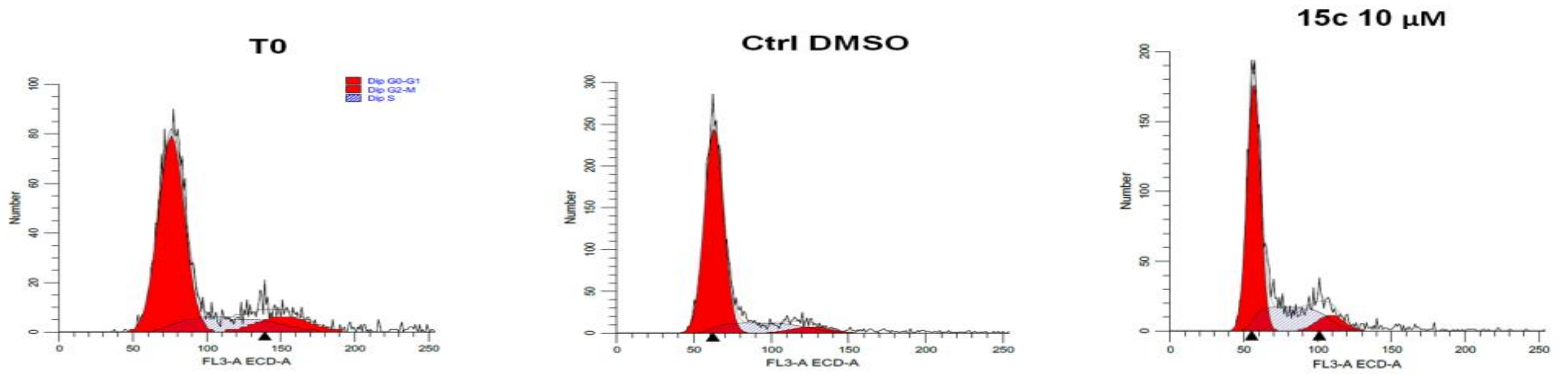


Ammazzalorso A. et al, under submission

Cell Cycle Analysis of MCF7 Cells



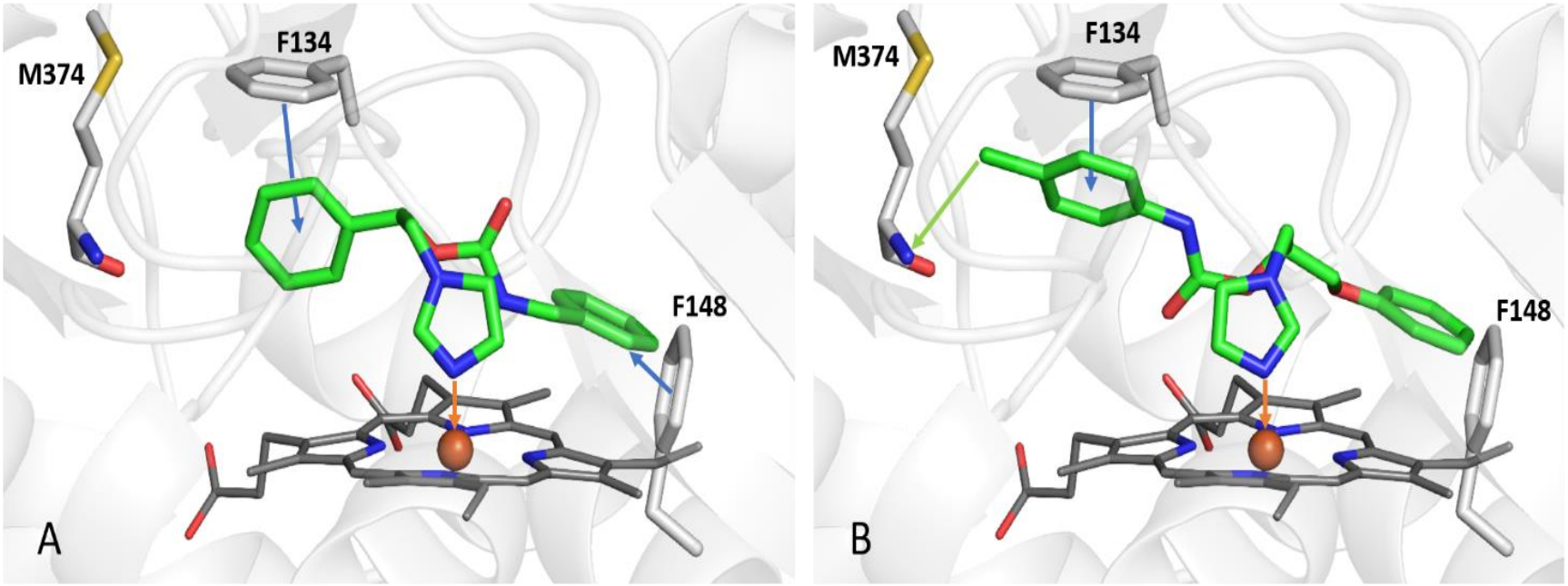




Ammazzalorso A. et al, under submission

Docking Studies

13a **15c**

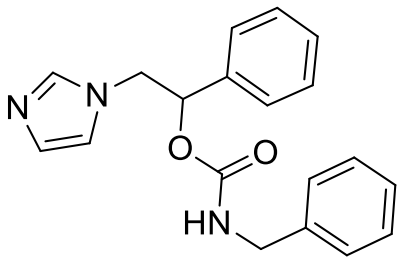


Zoomed in view of the binding site of aromatase. **13a** (Panel **A**) and **15c** (Panel **B**) are rendered in green stick representation. The most relevant residues are rendered as white sticks.

Ammazzalorso A. et al, under submission

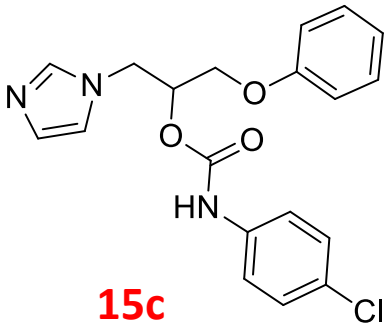
Conclusions

- A novel family of 22 azole-based aromatase inhibitors has been synthesized.
- Novel compounds were screened by an *in vitro* enzymatic assay against human aromatase, displaying submicromolar potency.
- Most active imidazole-based compounds were tested in MCF7 cells, in which they induced a marked reduction of cell viability and cytotoxic effects, producing a cell cycle block.
- Docking experiments confirmed for novel compounds a very similar binding mode with respect to letrozole.



13a

IC_{50} (aromatase) 0.82 μM
IC_{50} (MCF7) 6.58 μM



15c

IC_{50} (aromatase) 0.86 μM
IC_{50} (MCF7) 7.16 μM

Acknowledgments

G. d'Annunzio University
Chieti-Pescara

Medicinal Chemistry Unit

Rosa Amoroso, Barbara De Filippis, Marialuigia Fantacuzzi, Letizia Giampietro, Cristina Maccallini

Anatomy Unit

Amelia Cataldi, Marialucia Gallorini

Aldo Moro University
Bari

Medicinal Chemistry Unit

Orazio Nicolotti, Nicola Gambacorta