# Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXIX

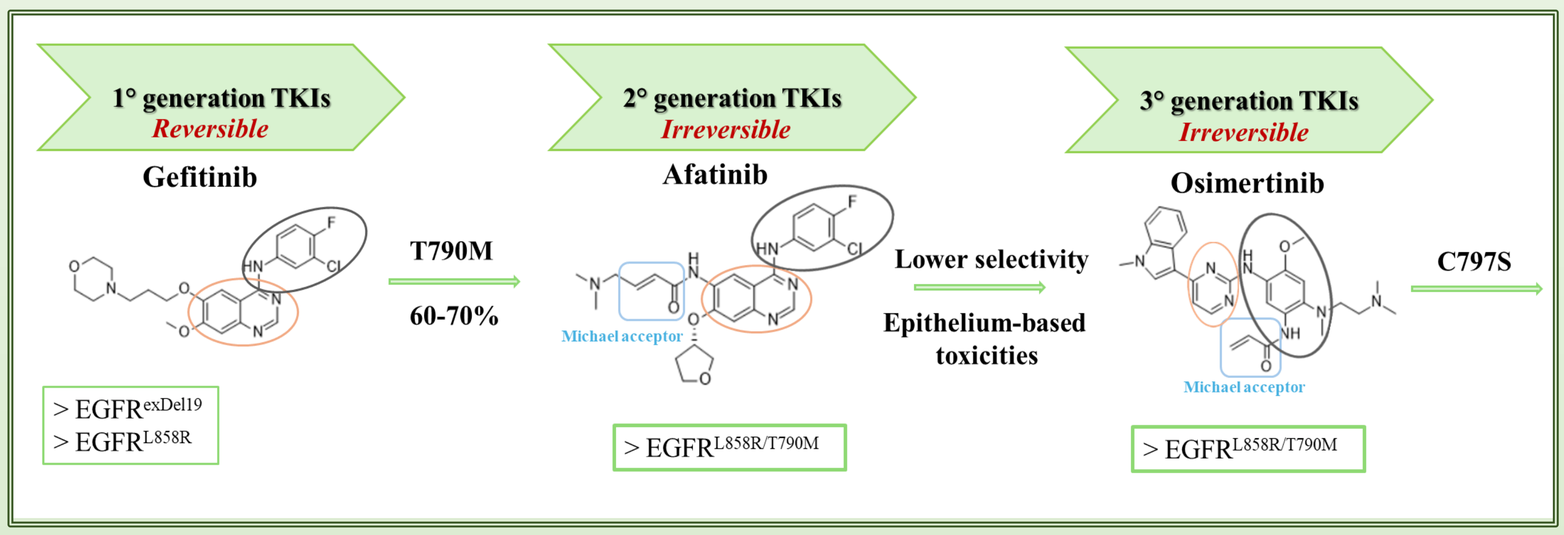
## HIT-TO-LEAD OPTIMIZATION OF FLAVONE-BASED TYROSINE KINASE INHIBITOR OF L858R/T790M EGFR ACTIVATION IN NSCLC


***Elena Romagnoli***

*DISVA, Laboratorio di sintesi e delivery di molecole bioattive* *Tutor: Prof.ssa Giovanna Mobbili*


## Background and purpose

The Epidermal Growth Factor Receptor (EGFR) is a membrane receptor which is activated with epidermal growth factor binding. EGFR is one of the most molecular targets in non-small lung cancer (NSCLC) where the mutant forms of EGFR are expressed. These forms are constitutively activated and determine cell proliferation, motility, invasion and chemio or radiotherapy resistance. The efficacy of Tyrosine Kinase Inhibitors (TKIs) could be compromised by additional mutantions in EGFR. The latest drug approved by the FDA is Osimertinib, which selectively and irreversibly inhibits EGFR $^{L858R/T790M}$, but the latest mutation (C797S) causes resistance to Osimertinib. The ongoing research aims to find new TKIs to overcome Osimertinib resistance.

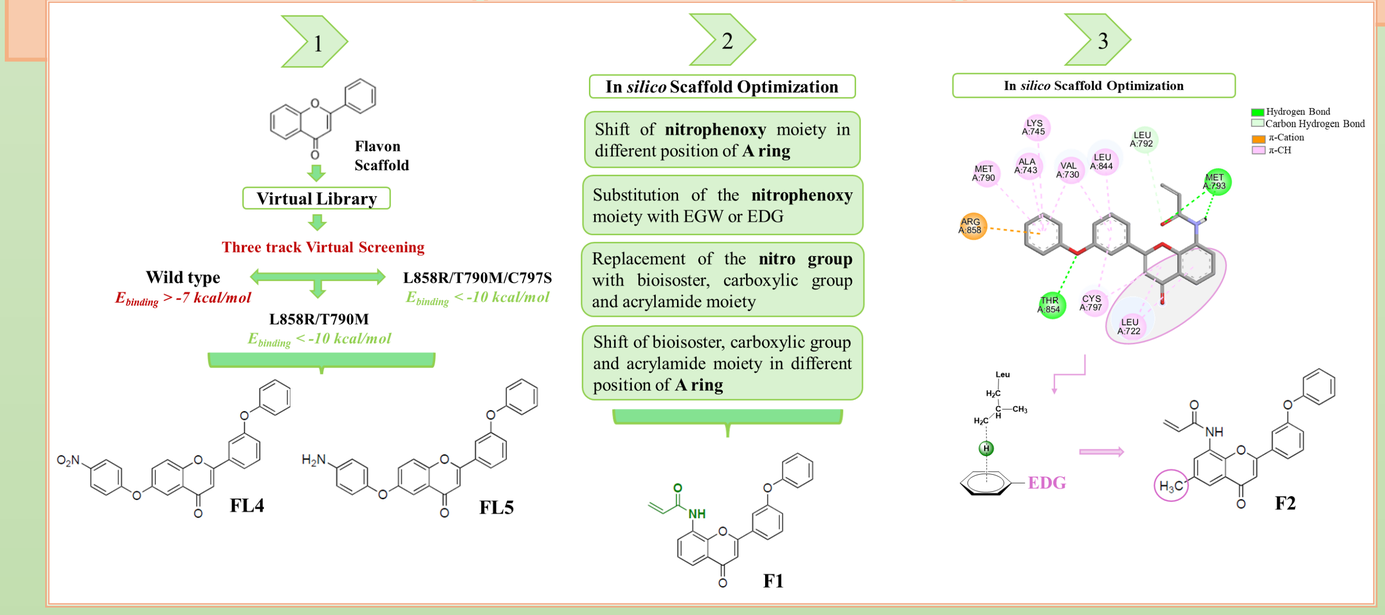



## Rational drug design and Enzyme Kinase Inhibition Assay

**1** The structure of the flavone has been chosen as scaffold for the *in silico* study of new possible derivatives. The modification of the scaffold has been rationalized in order to increase the affinity towards the mutant forms of the EGFR with respect to the wild type one; in particular, the interaction with the mutated residues has been considered.

**2** The optimization of hit compounds was developed with a second *in silico* study based on the semi-flexible docking approach. The F1 compound, which has the acrylamide group in position 8 of the ring A, is able to interact with both mutated residues of the bonding pocket. However, his Binding Affinity is still slightly higher than -10 kcal/mol.

**3** The presence of an EDG in position 6 of the ring A allows to strengthen the interaction π-CH between the ring A and the residue of leucine 722, lowering the binding energy ($E_{binding}$) to -10.2 kcal/mol. Attempts have been made to replace the acrylamide fragment with other amide substituents.

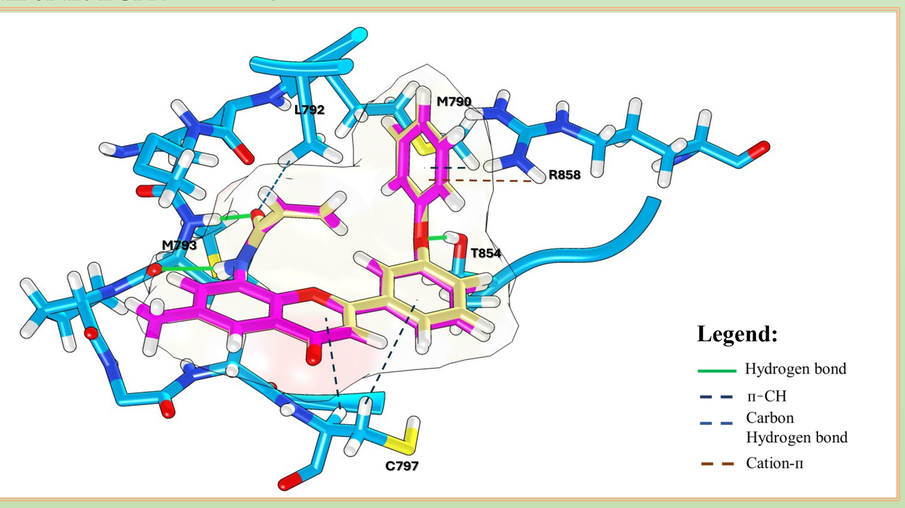



**Enzyme inhibition assay**
Recombinant EGFR proteins were produced at the Protein Facility, Structural Biology Lab, Elettra Sincrotrone Trieste.

**Table.** Calculated Binding Affinity and IC50 values of compound FL4, FL5, F2 and Osimertinib in complexes with mutant and wild type EGFR. Osimertinib was used as a control.

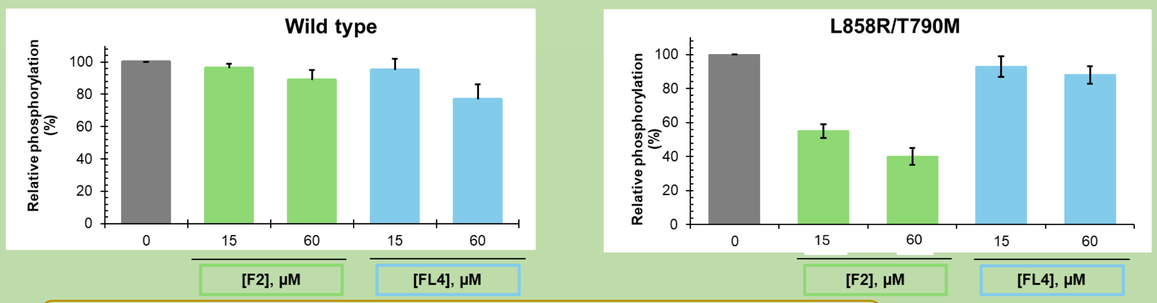
| | FL4 | | FL5 | | F2 | | Osimertinib | |
|---|---|---|---|---|---|---|---|---|
| | Wild Type | L858R/T790M | Wild Type | L858R/T790M | Wild Type | L858R/T790M | Wild Type | L858R/T790M |
| Binding Affinity (Kcal/mol) | -5,9 | -10,6 | -6,1 | -9,4 | -9,1 | -10,2 | -7,8 | -7,5 |
| IC50 (μM) | >150 | 12 | >150 | 39 | 25 | 0,1 | 5 | 0,2 |

**Figure.** Focused docking of **F1** (in khaki), and **F2** (in magenta) in the tyrosine kinase domain of the EGFR$^{L858R/T790M}$.




## Cellular Assays

**Table**. Ability of **F2** and **FL4** to inhibit the phosphorylation of EGFR Wild Type and L858R/T790M.







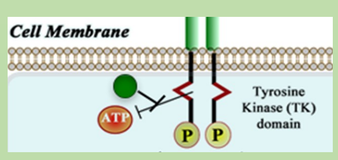



**Table.** Cytotoxicity in cancer cells.

| Cell lines | IC50 ± SD (μm) | | | Selectivity Index (SI)[b] | |
|---|---|---|---|---|---|
| | Erlotinib | FL4 | F2 | SI for **FL4** (wild type/mutant) | SI for **F2** (wild type/mutant) |
| **MCF7** low-expressing wt EGFR | 4 ± 3 | >100 | 60 ± 5 | 1,4 | 13 |
| **A549** high-expressing wt EGFR | 2,5 ±2 | >100 | 54 ± 3 | 1,4 | 12 |
| **H1975** EGFR L858R/T790M | 60 ± 4 | 72 ± 4 | 4,7 ± 1,2 | - | - |

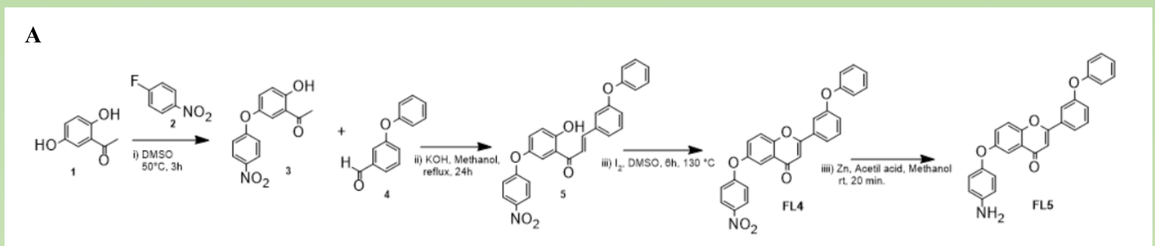
[b] SI, Selectivity Index (determined by the ratio between IC50 value MCF7 or A549 cancer cell lines and IC50 value in H1975 cancer line). A higher SI value corresponds to greater selectivity in inhibiting the mutated EGFR.

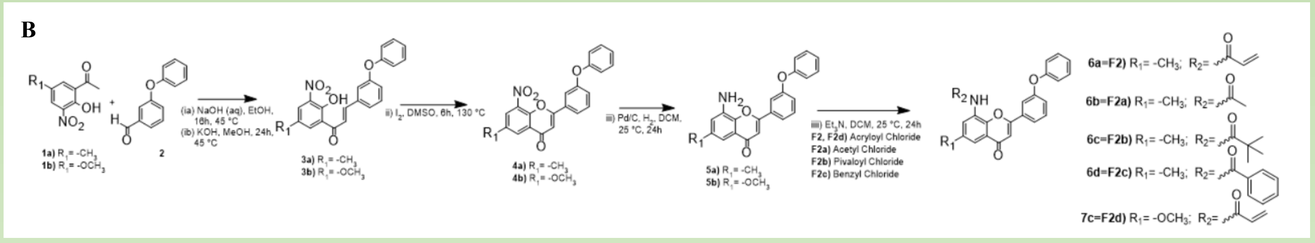
**Table.** IC50 values of F2 derivatives and Osimertinib in complexes with mutant EGFR. Osimertinib was used as a control. One-pot analysis with one concentration of Inhibitors (1 μM).

| Entry | Osimertinib | F2 | F2a | F2b | F2c | F2d |
|---|---|---|---|---|---|---|
| IC50 (μM) L858R/T790M | 0,087 | 0,1 | >10 | >10 | >10 | >10 |

## Synthesis of Flavon derivatives

**Figure.** A) Synthetic procedure of **FL4** and **FL5**, B) Synthetic procedure of **F2** and **F2 analogues**.




## Conclusions

The *in silico* study approach allowed to identify promising structures. The optimization process led to the identification of compound F2, which shows an IC50 value in the low nanomolar range in the enzymatic inhibition test. The substitution of the acrylamide group and the methyl group results in a significant loss of activity. We are considering the possible replacement of the $C_{sp2}$-$C_{sp2}$ double bond of the acrylamide group with the $C_{sp3}$-F bond since they have approximately the same length. This aspect could lead to the identification of a new compound with activity similar to or even greater than of F2.

## References


[1] C. Minnelli, E. Laudadio, G. Mobbili, R. Galeazzi, IJMS 2020, 21, 1721

[2] F. Teillet, A. Boumendjel, J. Boutonnat, X. Rono Med. Res. Rev. 2007, 28, 715-745

[3] C. Minnelli, E. Laudadio, et al. Bioorganic Chemistry 2022, 129, 106219