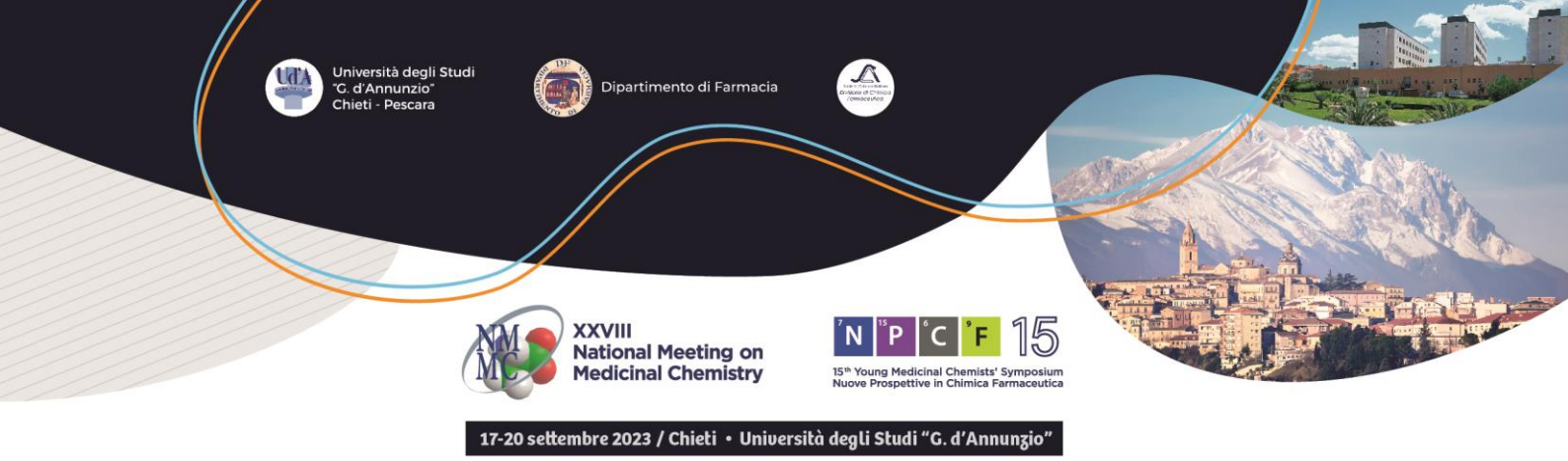

Please, indicate the preference between **oral, flash, or poster presentation:**

X Oral (20 minutes including the time for question)

☐ **Flash** (10 minutes including the time for question)

☐**Poster**

Please, indicate below both one Main topic and one or more Technologies for your abstract:

*Main topic: Cancer

*I Technology: Computer-aided drug design

II Technology: Molecular and chemical biology

III Technology: **Please select...**

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Deadline for submission of the Abstract: May 15st, 2023

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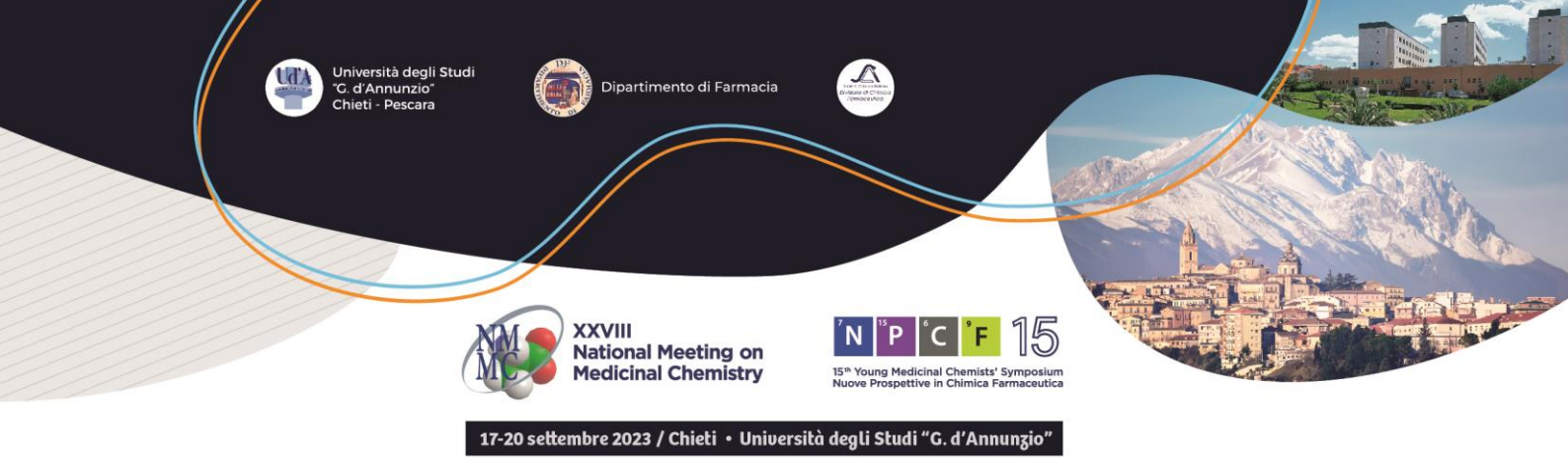

LEAD OPTIMIZATION OF HUMAN THYMIDYLATE SYNTHASE DIMER DISRUPTERS: FROM COMPUTATIONAL STUDIES TO EVALUATION OF THEIR BIOLOGICAL PROFILES

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Human thymidylate synthase is a homodimer of 76 kDa with two active sites formed by residues of both the monomers. This protein is involved in the conversion of dUMP to dTMP through a reductive methylation of the co-factor mTHF.[1] Due to hTS role, this enzyme has been used as target in cancer. Drugs like 5-fluorouracil (5-FU), Decitarbine and folates analogues like Raltitrexed are able to covalently bind the active site of the enzyme Due to their mechanism, there is an overexpression of the enzyme inside the cell leading to drug resistance. During the years, evidence have proven that hTS dimer is in equilibrium with its inactive monomers.[2] To overcome the drug-resistance, our research group has aimed to find new ways to interfere with this equilibrium to inactivate the enzyme without causing its overexpression by targeting the interface of the dimer shifting the equilibrium toward the monomers.[3] From a library of more than 150 compounds developed as new hTS inhibitors two molecules have been selected as lead for the optimization studies: E7 and AIC-A16. Aim of this optimization was increase the solubility, replacement of the toxicophore nitro (NO_2) with others EWG group like CF_3, increased activity on the recombinant protein and on cells.

Our optimization started with the analysis of the interactions between the leads and the proteins. To perform the computational studies, we used Maestro to perform docking simulations (Glide) and Induced-fit dockings (Glide+ Prime). We docked our leads in the peptide site of the x-ray crystal structure (3N5E). From the data obtained we designed a library of 22 new dimer disrupters that gave during simulation good to optimal scores >7.000 kcal/mol. The selected compounds have been synthetized, purified (HPLC purity >99%) and characterized with good yields (20-95%). All compounds have been tested spectrophotometrically against hTS to evaluate the Ki (Ki 1-10 µM) on the recombinant protein and the effects of compounds on cell growth has been determined by the crystal violet dye assay on HT29 and HCT116 cell lines of colorectal cancer.

The results obtained by this optimization process have shown that we successfully increased the activity on cells compared to the lead AIC-A16 in some cases to 60 % of growth inhibition. We have successfully removed the nitro group leading to more active compounds than the lead. In conclusion, we succeed in the optimization of the selected lead.

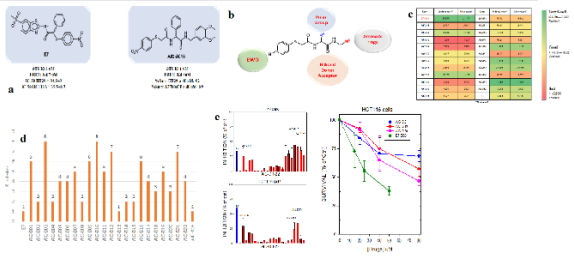

Figure 1, a. Structure and biological profiles of Leads. b. Site of modifications. c. Docking score heat map. d. Ki values for AIC-D. e. growth inhibition % on HT29 and HCT 116.

References

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3. Costantino, L. et al. Destabilizers of the Thymidylate Synthase Homodimer Accelerate Its Proteasomal Degradation and Inhibit Cancer Growth. Elife 2022, 11, 1–57.