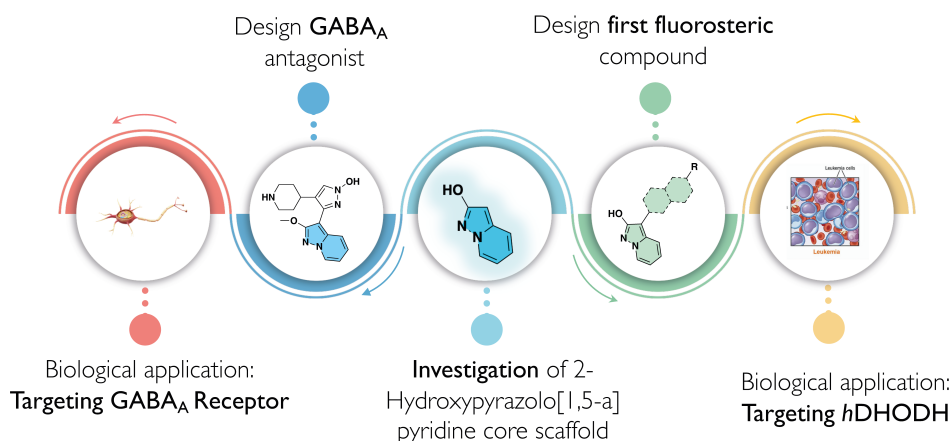


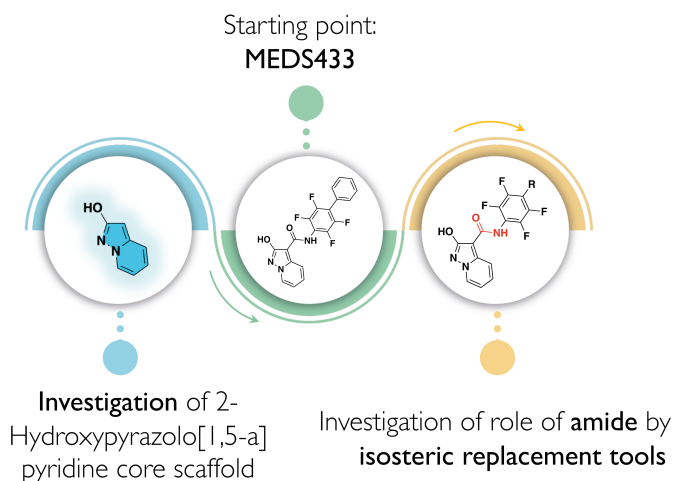
# Abstract

*Fluorescence imaging* is a widely tool used in biology to study and visualize biological processes in living organisms. Over the last ten years, several *fluorescent probes* have been investigated and developed with the ultimate goal of being used for fluorescent imaging studies. While designing probes owing high affinity for a specific target (the so-called *receptor-target fluorescent probe* technique) several problems can arise. Considering that usually targets are extremely sensitive to alterations of the structural features of their ligands, the modulation of small molecules with a fluorescent dye quite often result in reduced affinity. In this occasion, we decided to develop the first *fluorescent isostere of carboxylic acid*, a tool that can be used in the design of *intrinsically fluorescent ligands*. We nicknamed it *fluorostere*, a term obtained by merging two words *fluorescent* and *isostere*. Starting from pyrazolo[1,5-a]pyridin-2-ol, a small heterocyclic used in the (bio)isosteric substitution of carboxylic acids, we have systematically modulated its structure in order to obtain an isostere with an optimized fluorescence profile.



**Figure A.** Investigation of fluorescent properties of pyrazolo[1,5-a]pyridin-2-ol for the design of new *intrinsic fluorescent bioactive compounds* targeting *hDHODH* and GABA<sub>A</sub> receptor.

To provide a first proof of concept of the application of this new technology, the knowledge gained for the design of intrinsic fluorescent ligands was applied to the investigation two biological targets that have been intensively studied in the IT-DK groups: *h*DHODH and the GABA<sub>A</sub> receptor (Figure A). Regarding *h*DHODH, the technology enabled the development of a novel *fluorosteric* inhibitor, which after synthesis and characterization, showed inhibitory activity in the nM range. In addition, a co-localization study with *Red-Mitotracker*, a fluorescent probe generally used to visualize mitochondria (where the biological site of *h*DHODH is located), revealed the compound's ability to reach the target enzyme at the cellular level. For the study of GABA<sub>A</sub> receptors, the design and partial characterization of two possible fluorescent compounds were here presented. The investigation for their fluorescent and pharmacological properties will be completed in the near future.



**Figure B.** Investigation of the amide moiety of **MEDS433** by isosteric replacement tools.

In this dissertation, a sideline was also dedicated to the optimization **MEDS433**, a potent *h*DHODH inhibitor containing the pyrazolo[1,5-a]pyridin-2-ol substructure developed in the UniTo group (Figure B). The structure of

**MEDS433** is characterized by the presence of this heterocycle linked to a biphenyl structure by an *amide moiety*. Since examination of the crystallographic structure of **MEDS433** revealed that this amide moiety does not appear to be a pharmacological element, several classical and non-classical isosteres were here presented to thoroughly investigate the role of this amide, and characterized up to the profile of cellular activity against acute myeloid leukemia (AML) cell lines.