ELTE-x: A multidisciplinary approach for the synthesis of a new family of potent FKBP12 inhibitors

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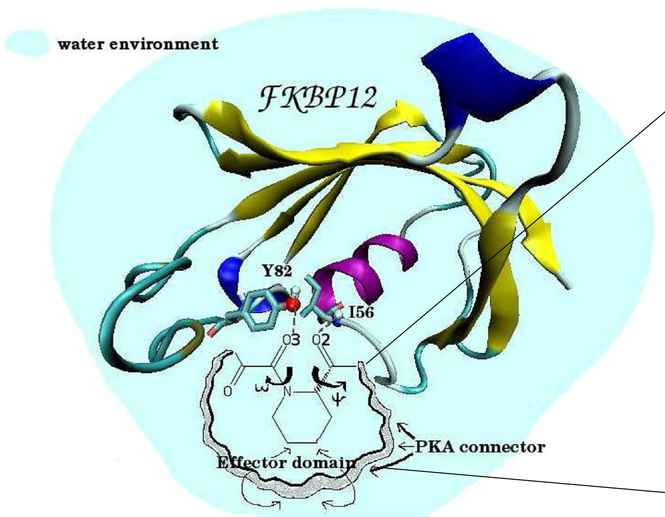
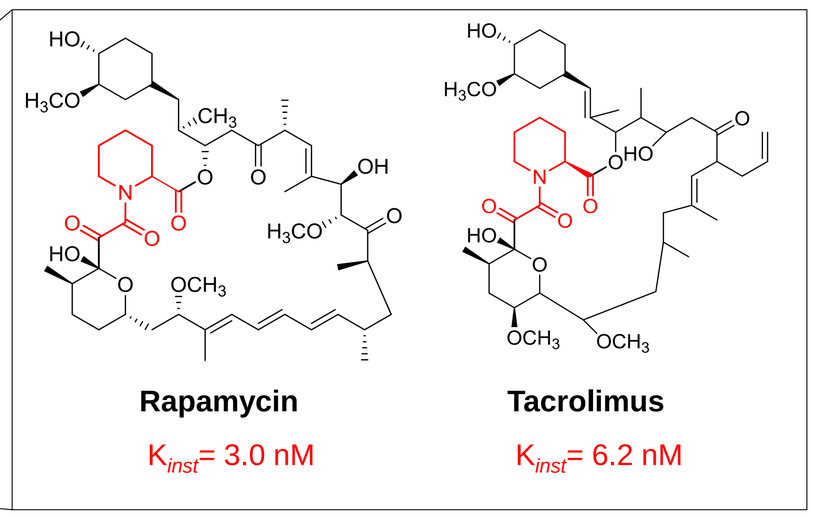
Pharmacore=PKA= pipecolyl α-keto amide

Starting point

FKBP12 protein

FKBP12, the smallest member of the family of FKBP immunophilins, has a central role in immunosuppression and cell proliferation due to its specific peptidyl-prolyl isomerase (PPI) function.





FKBP12 in humans binds the immunosuppressant macrolides Tacrolimus (FK506) and Rapamycin with nanomolar activity.

In literature, a huge number of FKBP12 inhibitors are reported. Many of them show poor activity despite the synthetic efforts required for their preparation.[1]

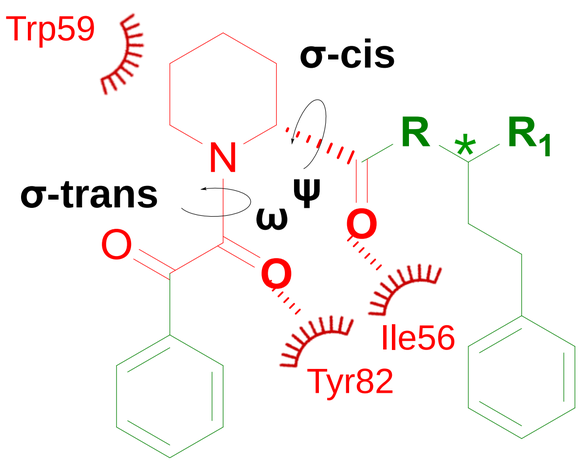
First step

***In silico* simulation**

We defined the pharmacore requirements for potential synthetic FKBP12 inhibitors, based on preliminary costless in silico simulations and conformational analysis.[2]

The essential ingredients of the pharmacore for the PPI domain

REM computational studies identified the **green portion** suitable for an optimal binding conformation.



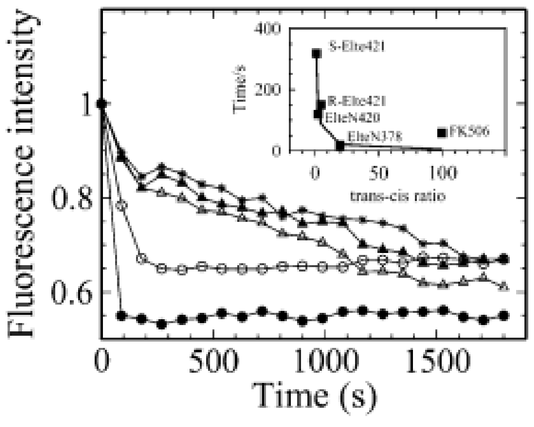
HYDROPHOBIC INTRALIGAND interactions impart to the ligand a quasi cyclic structure mimicking the rigidity of the macrolides in a water environment and correctly exposing the two binding carbonyl units of the PKA.

POLAR PROTEIN-LIGAND interactions are regulated by the two angles **ω** and **ψ**, thus imparting optimal mutual exposure of the two binding carbonyls.

Second step

Synthesis

We set up a rational drug design organic synthesis to realize a new family of nanomolar FKBP12 inhibitors.[3]

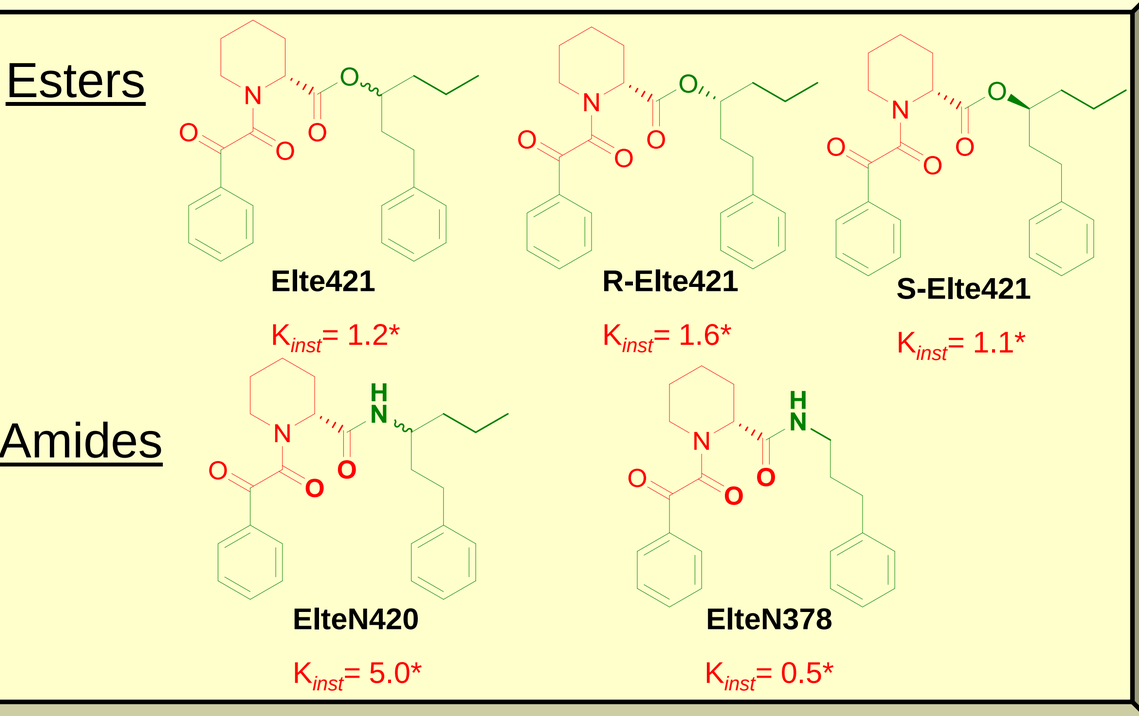


Inhibitors binding with the FKBP12 protein causes an intrisic fluorescent quenching of **Trp59** residue in the active site.

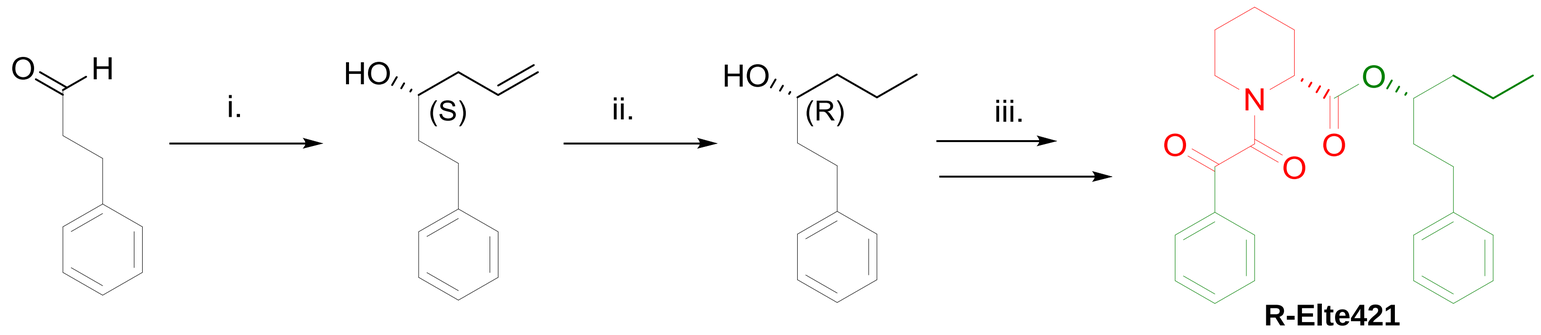
Third step

Fluorescent quencing

ELTE-x family



***The experimental binding affinity K_{inst} versus FKBP12 is expressed as the ratio with respect to that of the FK506 compound measured in the same experimental conditions.**



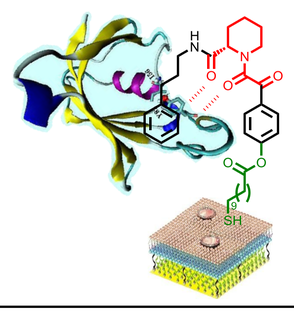
R-Elte421

Synthesis of enantiopure **R-Elte421**: All the other FKBP12 inhibitors were similarly prepared.

i. (a) (−)-β-allyldiisopinocamphenylborane (1 equiv), Et_2O, −100 °C−rt; (b) 3 M NaOH (3.5 mL), 30% H_2O_2 (1mL), reflux, 1 h. ii. H_2, Pd/C 5% (0.1 equiv) in MeOH, rt, 2.5 h. iii. …..ask Elte.

In progress…

Using the skeleton of the ElteN378, we planned suitable synthetic modifications for the assembling of a sensor for FKBP12. Again, *in silico* calculations suggested the position where to insert the thiolic linker without affecting the interaction of PKA with the protein.



A FKBP12 sensor can find applications as biomarker for early diagnosis in AD or PD and be used for assessing the role of FKBP12 protein in α-synuclein aggregation as a model for amyloidogenesis in biomimetic systems.

1. Holt, D.A.; Luengo, J.I, et all *JACS,* **1993**, 115, 9925-9938

2. Bizzarri, M.; Tenori, E.; Martina, M. F.; Marsili, S.; Menichetti, S.; Caminati, G.; Procacci, P. *J. Phys. Chem. Lett.*, **2011**, *2*, 2834–2839

3. Martina, M. F.; Tenori, E.; Bizzarri, M.; Menichetti, S.; Caminati, G.; Procacci, P. *J. Med. Chem.,* **2013**, *56*, 1041-1051