UNIVERSITÀ DEGLI STUDI DI MILANO
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ELAGOLIX ANALOGUES AS POTENTIAL NEW GnRH ANTAGONISTS: DESIGN, SYNTHESIS AND CHARACTERIZATION

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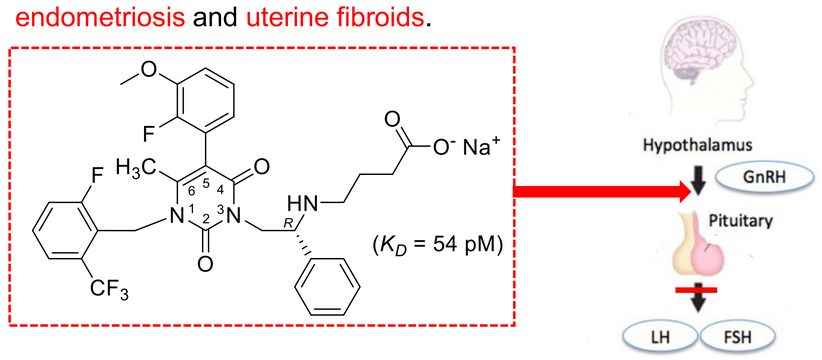
BACKGROUND

ELAGOLIX: the first non-peptide GnRH antagonist

Approved by FDA for sex-hormone dependent diseases such as endometriosis and uterine fibroids.



Oral bioavailability

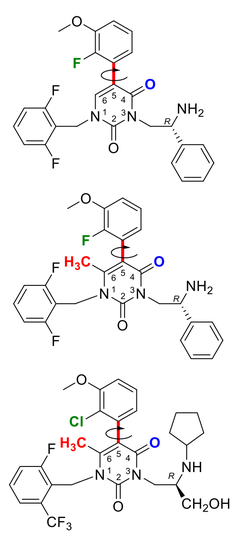
Pros

- Fast onset and offset
- Better patient compliance
- Better safety profile

ATROPISOMERISM vs ACTIVITY

The interaction of the *ortho*-substituent of the 5-aryl group with the 6-CH_3 and with the electronegative oxygen atom of C=O at 4-position causes a slow rotation around the C-C bond between the two rings.

TORSION ROTATIONAL ENERGY BARRIER



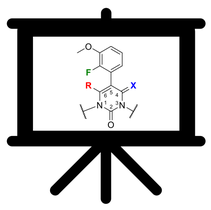
FREE ROTATION
Ki = 5.3 nM
(Without atropisomerism the activity decreases)

SLOWLY INTERCONVERTING ATROPISOMERS
Ki = 0.56 nM

STABLE ATROPISOMERS
Atropisomer *S* *Ki* = 190 nM
Atropisomer *R* *Ki* = 0.30 nM
(Stereo-preference for receptor interaction)

AIM
New analogues:
MODIFICATIONS AT
4- and/or **6**-POSITION?

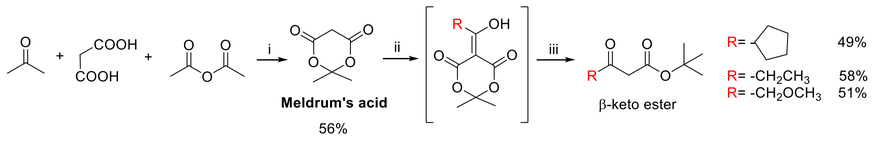
NOT YET EXPLORED!



MODIFICATION AT 6- POSITION

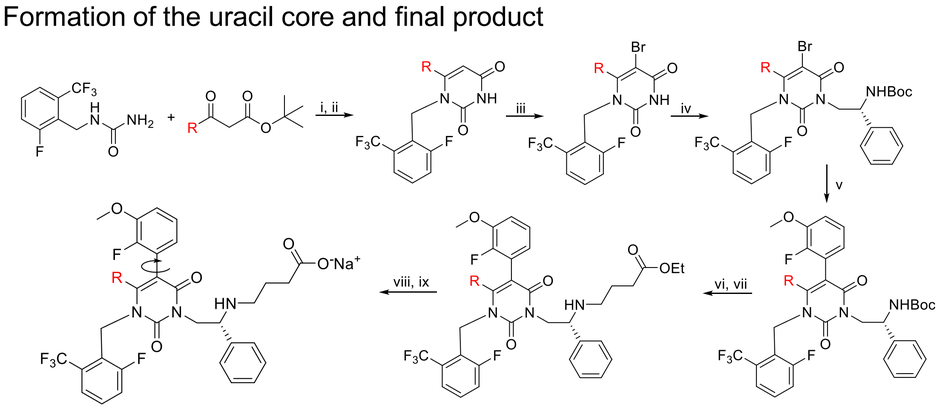
What happen introducing bulkier groups than CH_3?

1) Synthesis of the suitable β-keto ester



Reagents and conditions: i. H_2SO_4 conc., O°C, N_2; ii. RCOCl, py, CH_2Cl_2; iii. *t*-BuOH, toluene, reflux.

2) Formation of the uracil core and final product

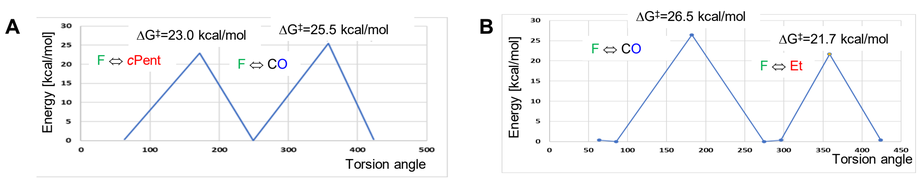


Reagents and conditions: i. Toluene, reflux, Dean-Stark apparatus; ii. *p*-TsOH; iii. Br_2, AcOH; iv. (*R*)-(-)-2-(Boc-amino)-2-phenylethanol, PPh_3, DEAD, THF; v. 2-fluoro-3-methoxyphenylboronic acid, $Pd(PPh_3)_4$, H_2O/1,4-dioxane, reflux, Ar; vi. TFA, CH_2Cl_2; vii. ethyl 4-bromobutyrate, DIPEA, DMF, 60°C; viii. NaOH, H_2O, EtOH; ix. extraction with MIBK followed by precipitation from *n*-heptane.

Compound	R	Overall yield	Interconversion time of atropisomers*
Elagolix	-CH_3	21%	< 17h
1		8%	≈ 9 days
2	-CH_2CH_3	10%	≈ 24h
3	-CH_2OCH_3	8%	≈ 24h

- Evaluated by chiral HPLC.
- The atropisomers were separated by semi-preparative chiral HPLC and stored in IPA/Hexane 8:2 solution.

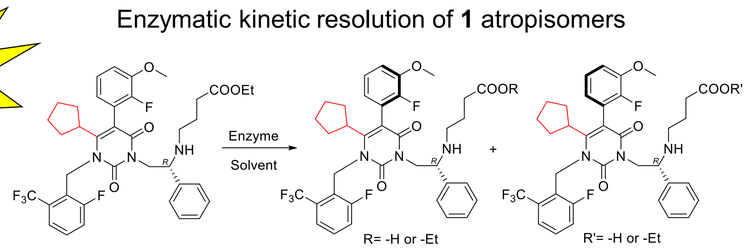
QM Gaussian16 calculations, B3LYP/6-31G(d,p) level, vacuum, of **1** (**A**) and **2** (**B**) atropisomers



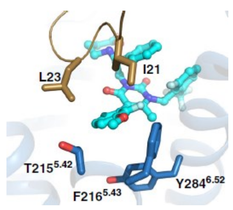
The modification at the 6-position affects both the energy barriers between the alkyl group and fluorine, and between fluorine and oxygen through inductive effect within the uracil ring.

Work in progress

Enzymatic kinetic resolution of **1** atropisomers



***h*GnRH1R-elagolix complex**



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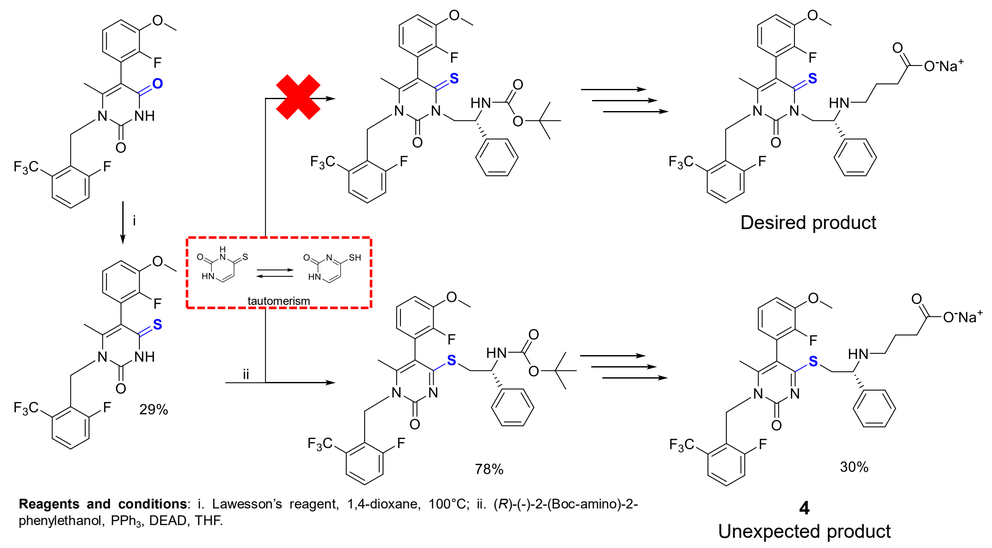
Computational studies

Organic chemistry

MODIFICATION AT 4- POSITION

What happen replacing the oxygen?



Reagents and conditions: i. Lawesson's reagent, 1,4-dioxane, 100°C; ii. (*R*)-(-)-2-(Boc-amino)-2-phenylethanol, PPh_3, DEAD, THF.

Serendipity

The unexpected compound **4** has:

❑ atropisomers interconversion time: 3 days;

❑ Molecular Dynamics simulations showed:

- ΔG value (ΔG= -97.0 kcal/mol) comparable to elagolix (ΔG= -97.6 kcal/mol)
- different binding pose at the active site

ACHIEVEMENTS and FUTURE PERSPECTIVES

✓ New elagolix analogues differently substituted at the 4- and/or 6-position of the uracil moiety were designed and synthesized with the support of molecular modelling techniques.

✓ Compounds **1** and **4** present higher atropisomers interconversion time than elagolix.

The research work will proceed with:

➢ the separation of atropisomers by means of biocatalytic approaches;

➢ the investigation of the biological properties using HEK 293 cells expressing the human GnRH receptor;

➢ the design and synthesis of novel analogues characterized by higher steric hindrance at the 6-position of the uracil moiety.