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Selective inhibition of the iNOS by acetamidine derivatives

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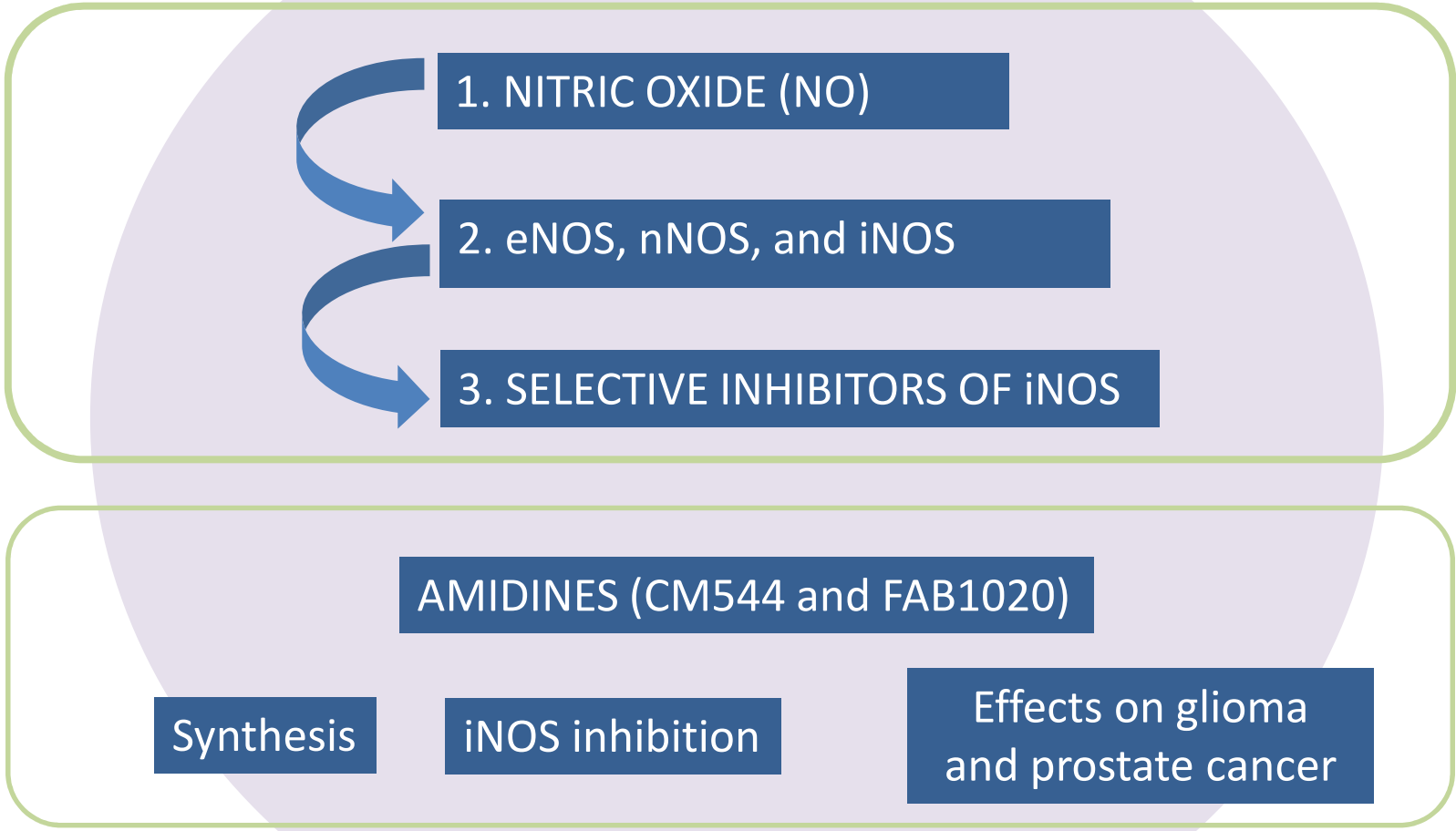
Graphical Abstract

Selective inhibition of the iNOS by acetamidine derivatives

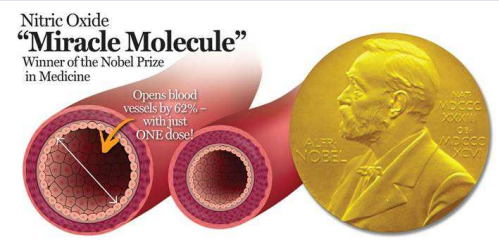


Abstract: Nitric oxide (NO) is a small radical which plays a key role in various physiological as well as pathological processes in mammals, bacteria, and plants. It is produced by enzymatic conversion of the natural substrate L-Arg, catalyzed by Nitric Oxide Synthases (NOSs), a family of three isoenzymes, including the constitutively expressed neuronal NOS (nNOS) and endothelial NOS (eNOS), and the inducible iNOS. This latter isoform is extensively expressed during inflammation, promoting a sustained generation of NO and reactive nitrogen species, responsible for the damage of a wide variety of biomolecules, including nucleic acids, proteins and lipids.
In the last years, we have designed and synthetized several acetamidines that revealed to be potent and selective inhibitors of both iNOS and nNOS. Docking studies showed that the acetamidine moiety anchors the inhibitor to the Glu and Trp into the NOS catalytic domain, while other groups extend in the substrate access channel, where are localized the major differences between NOS isoforms. These specific interactions are responsible for the observed activity and selectivity.
In this presentation, the development of two iNOS inhibitors containing the acetamidine moiety will be discussed, starting from the synthesis and evaluation of the inhibitory iNOS activity, up to the description of some biological tests in vitro.

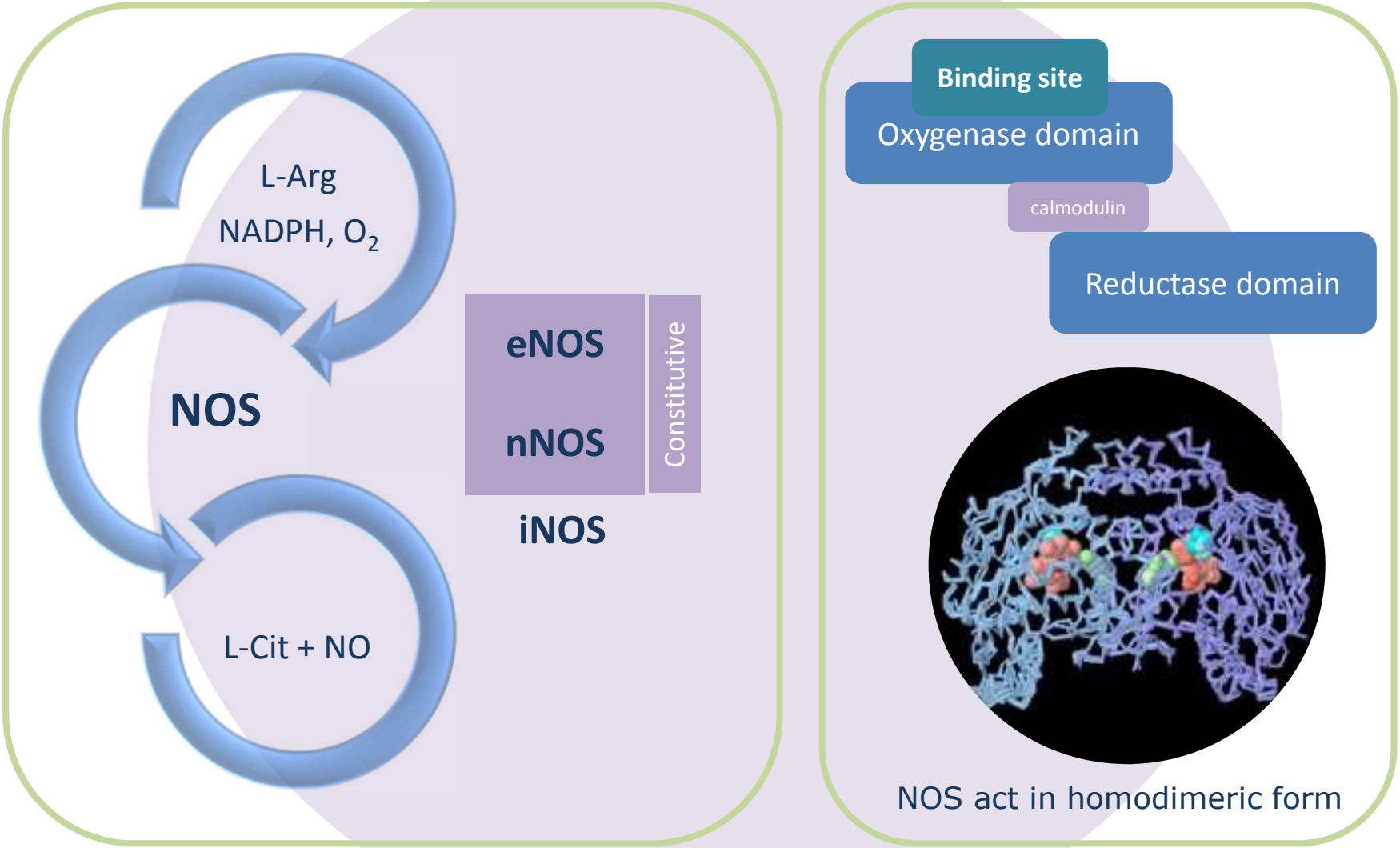
Keywords: Nitric oxide; NOS inhibitors; acetamidines

NITRIC OXIDE (NO)

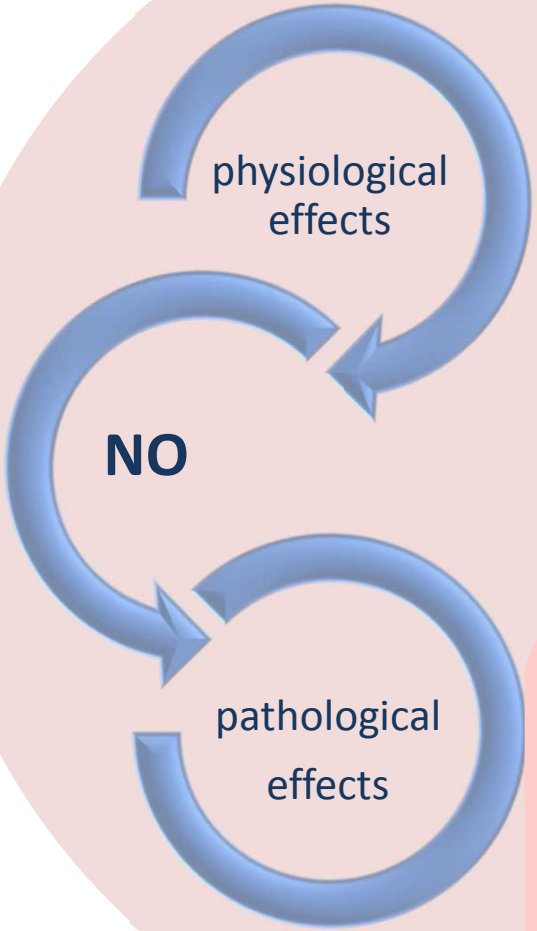
- Colorless gas abundant in the body, unstable, short-lived, and renewed continuously
- It carries messages at the cellular level, and it is able to penetrate cellular membranes because of its gaseous structure
- Nobel Prize in 1998 as "*miracle molecule*"



NITRIC OXIDE SYNTHASES (NOSs)



Introduction



Vasodilatation
Neurotrasmission
eNOS and nNOS

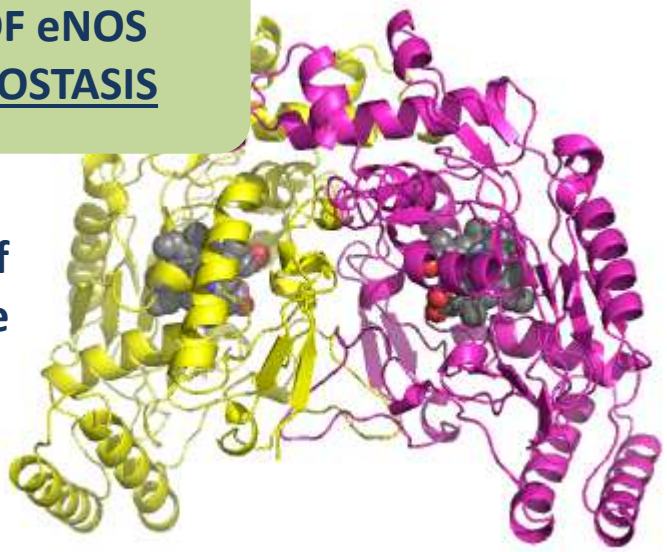
Inflammation
Cancer
iNOS
Reactive Nitrogen Species (RNS)

The dysregulated production of the NO by iNOS is involved in the malignancy of different cancers, as well as in chemoresistance, due to the activation of several signaling mediators
NO inhibition can be considered an emerging therapeutic possibility to treat these diseases

INHIBITORS OF iNOS

SELECTIVE INHIBITION OF iNOS
THERAPY OF CANCER

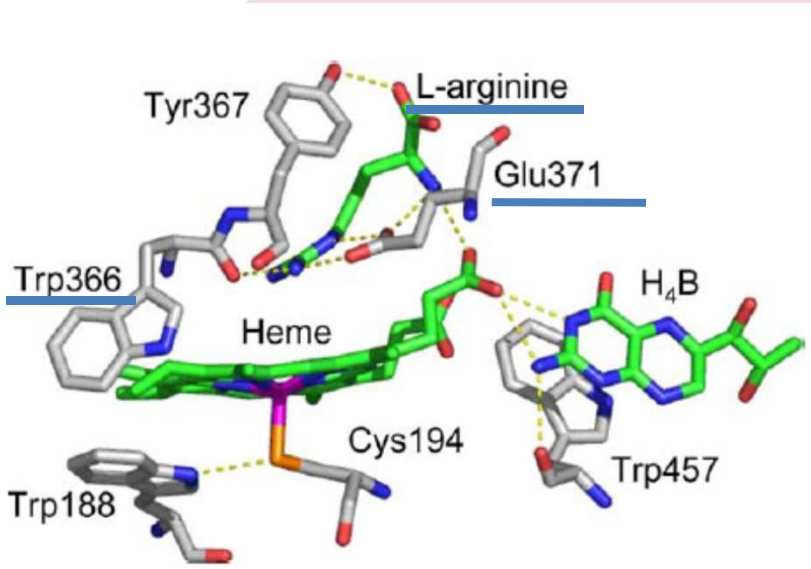
INALTERATED ACTIVITY OF eNOS
CARDIOVASCULAR HOMEOSTASIS



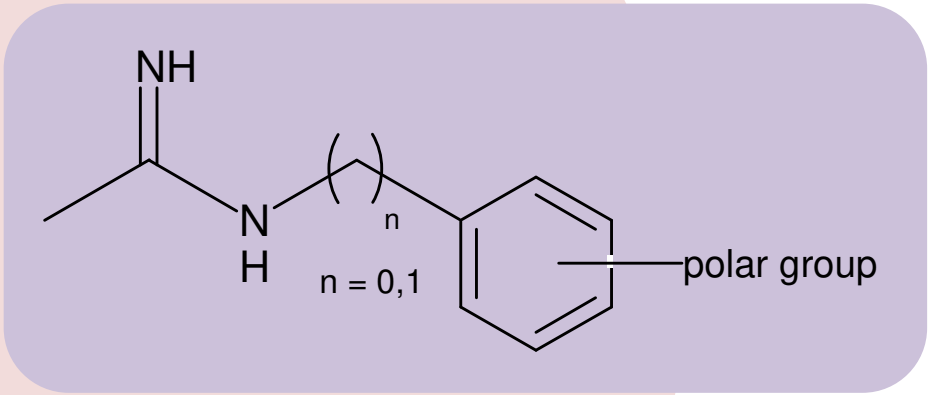
High degree of conservation of oxygenase domain for the three isoforms

Small differences were highlighted

SELECTIVE INHIBITORS OF iNOS: ACETAMIDINES

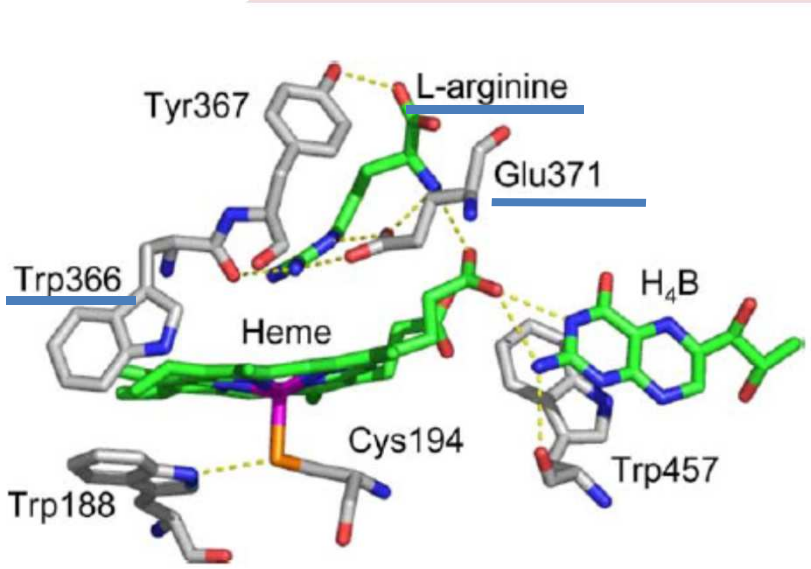


L-Arg in the iNOS active site

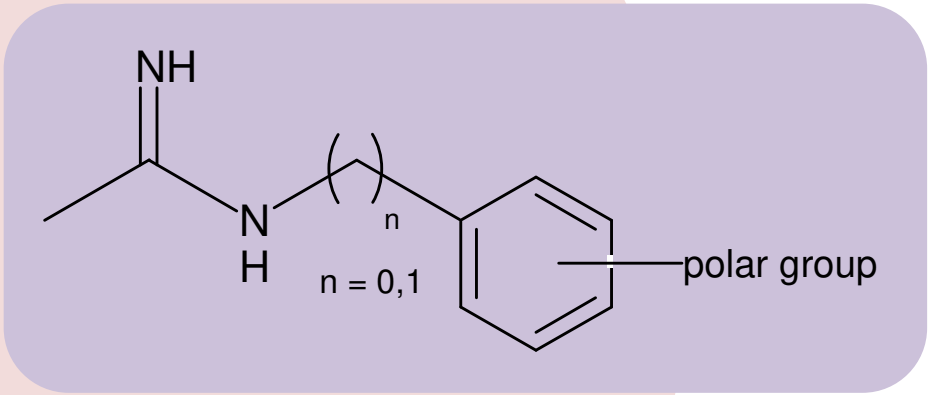


Hydrogen bonds between the acetamidine and Glu and Trp located in the iNOS oxygenase domain

SELECTIVE INHIBITORS OF iNOS: ACETAMIDINES

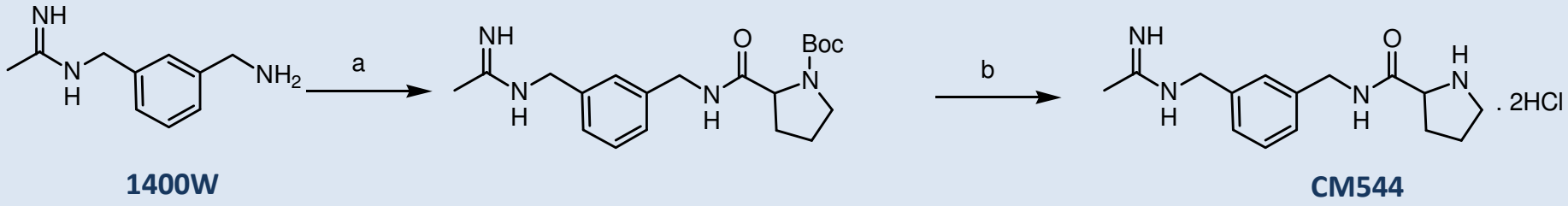


L-Arg in the iNOS active site



Hydrogen bonds between the acetamidine and Glu and Trp located in the iNOS oxygenase domain

CM544: SYNTHESIS AND iNOS INHIBITION



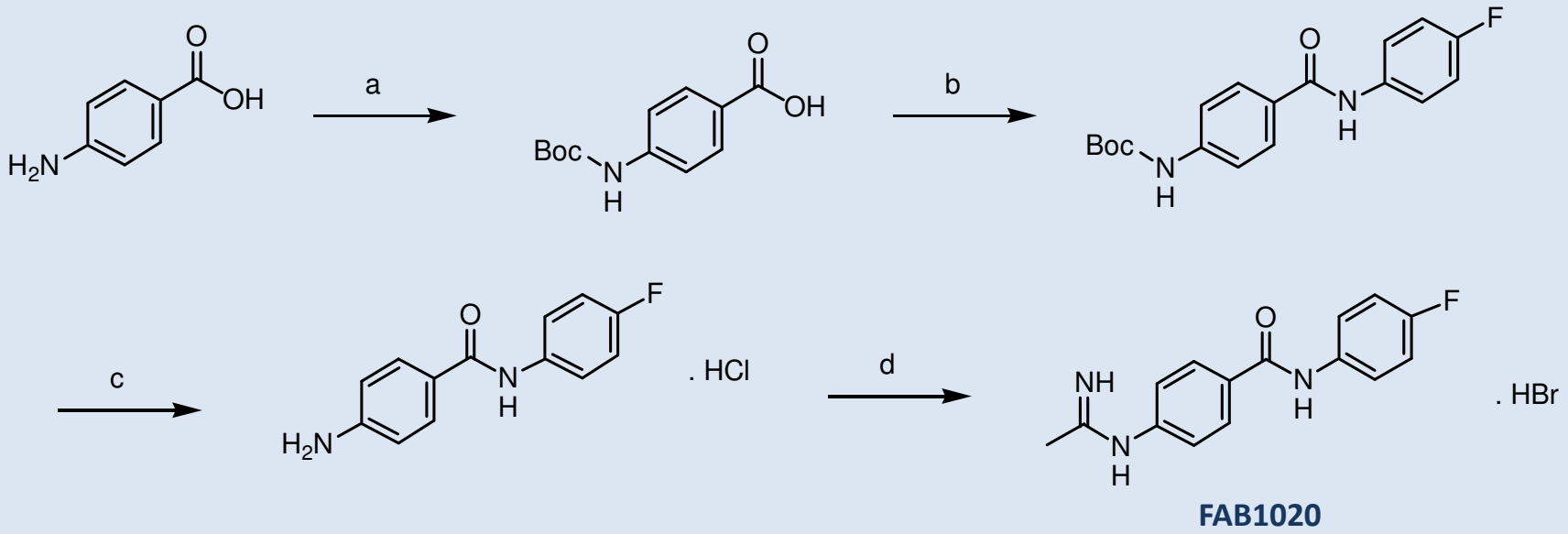
1400W **CM544**

a) Boc -Pro, *i*BuOCOCl, NMM, dry DMF, N_2, -15 °C, 24 h; b) HCl 4 N, dioxane, r.t., 24 h

IC_{50} (μM)			Selectivity	
eNOS	nNOS	**iNOS**	iNOS/nNOS	**iNOS/eNOS**
265	> 10	**0.058**	> 172	**4569**

Results and discussion

FAB1020: SYNTHESIS AND iNOS INHIBITION



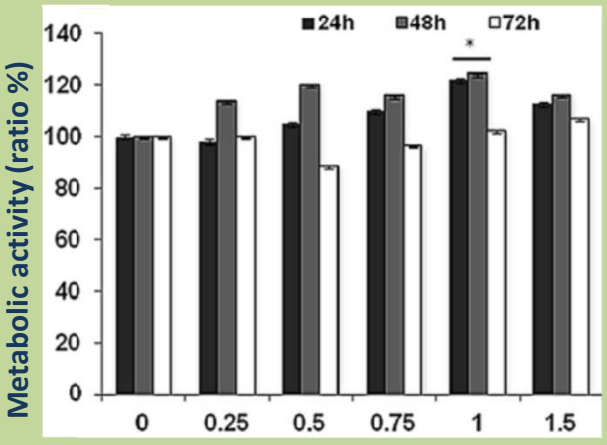
a) $(Boc)_2O$, dioxane, NaOH 1N, 0 °C to r.t., 24 h; b) isobutylchloroformate, dry DMF, N_2, -15° C to r.t., 24 h; c) HCl 4N, dioxane, r.t. 24 h, e) *S*-naphtyl-thioacetimidate hydrobromide, $CHCl_3$, b.t. 24-48h

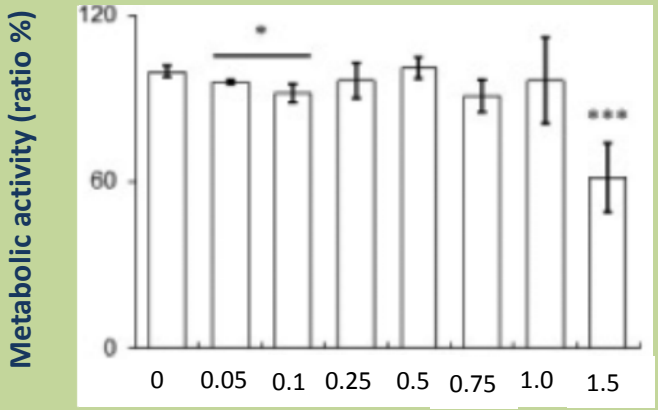
IC_{50} (mM)			SELECTIVITY	
nNOS	iNOS	eNOS	iNOS/nNOS	iNOS/eNOS
6.2	0.011	> 10	563	> 900

Results and discussion

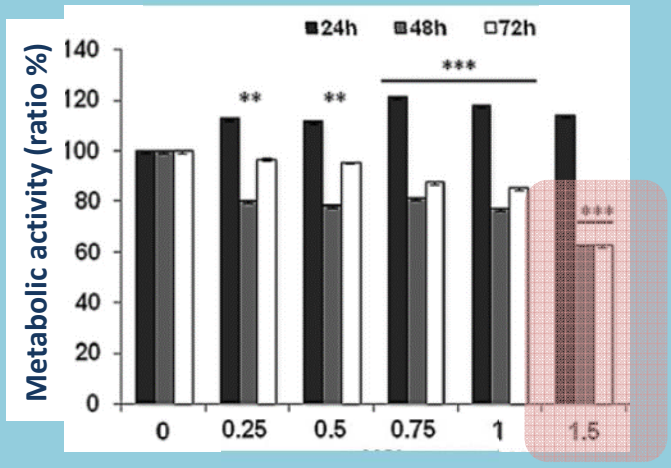
Glioma

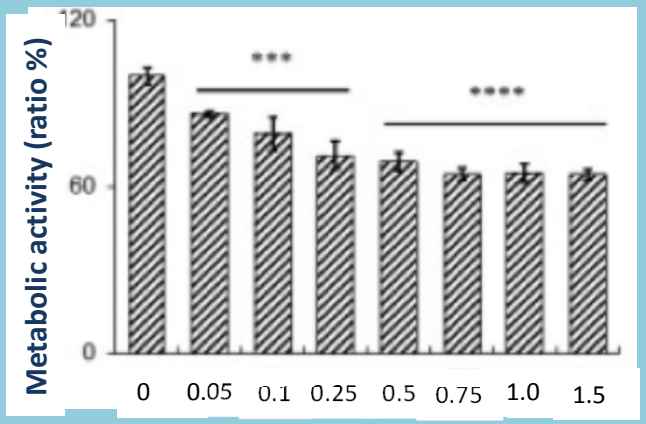
RAT ASTROCYTES (CTX/TNA2)





C6 RAT GLIOMA CELLS





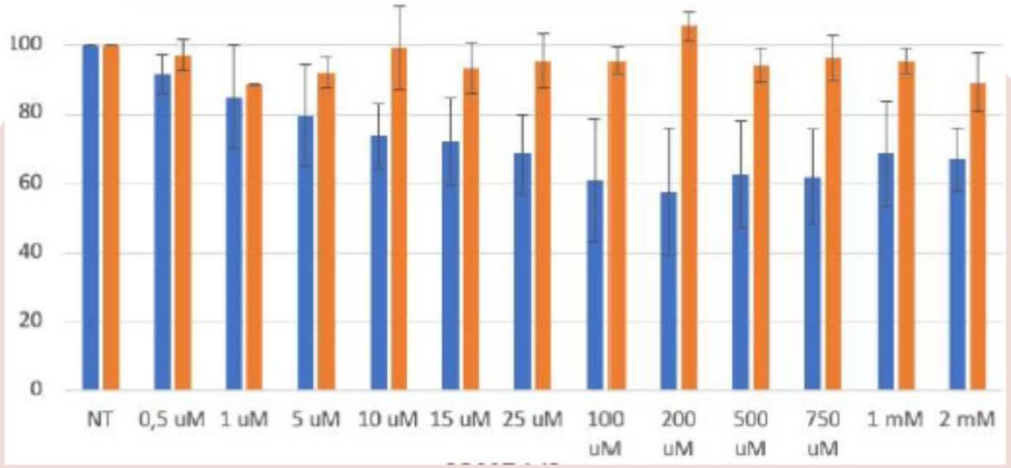
Results and discussion

PROSTATE CANCER

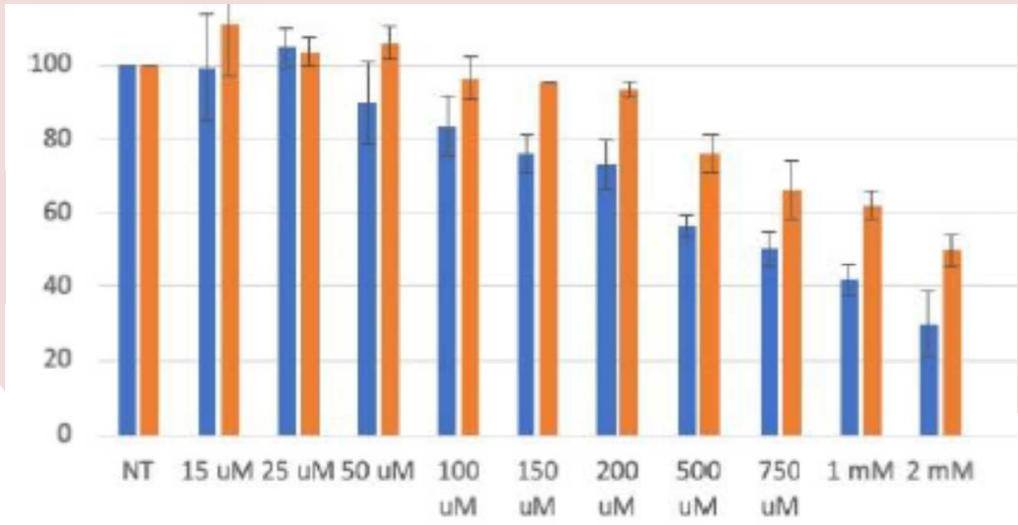
PNT1A: non tumoral cells

PC3: prostate cancer cells

CM544



FAB1020



Conclusions

In this presentation, we investigated the acetamidine-based iNOS inhibitors CM544 and FAB1020

For both compounds, the synthesis, the selective inhibitory activity against iNOS, and the evaluation of antiproliferative effects on rat glioma and prostate cancer cell lines were presented

CM544 and FAB1020 were found potent inhibitors of iNOS with high selectivity over the constitutive isoforms

The antiproliferative effects are in accordance with NOS selectivity data, being two acetamidines active on tumor cells which overexpress iNOS as a marker of malignancy

FAB1020 seems to be more potent than CM544. This effects could be attributed to its increased lipophilicity

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