SYNTHESIS AND *IN VITRO* BIOLOGICAL EFFECT OF GNRH - PROTOPORPHIRIN IX CONJUGATES

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GnRH

Gonadotropin-releasing hormone (GnRH) receptors are overexpressed on various tumor cells.
Primary biological function of GnRH is the regulation of the gonadal activity and the vertebrate reproduction[1]

GnRH-I
native ligand of the GnRH receptors, that is synthesized and released in the hypothalamus

Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

increases receptor binding affinity and stability[2] — Lys(Bu) *D*Lys — *proper conjugation site[3] (superagonist)*

GnRH-II
neuromodulator and sexual behavior stimulator; originally isolated from chicken hypothalamus

Glp-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2

Lys(Bu) *D*Lys

GnRH-III
native isoform of the human GnRH isolated from sea lamprey

Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2

Lys(Bu) Asp(OMe) *no amide bond formation*

Synthesis on Fmoc-Rink Amide MBHA resin by SPPS

Glp-His(Trt)-Trp-Lys(Dde)-Tyr(*t*Bu)-*D*Lys(Boc)-Leu-Arg(Pbf)-Pro-Gly-R

↓ Dde cleavage (2% hydrazine / DMF) butyric anhydride, DIPEA in DMF

Glp-His(Trt)-Trp-Lys(Bu)-Tyr(*t*Bu)-*D*Lys(Boc)-Leu-Arg(Pbf)-Pro-Gly-R

↓ 95% TFA + 2.5% H_2O + 2.5% TIS

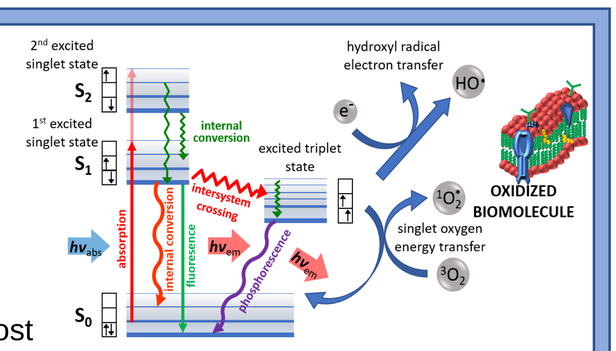
Glp-His-Trp-Lys(Bu)-Tyr-*D*Lys-Leu-Arg-Pro-Gly-NH_2 = GnRH-I[^{4}Lys(Bu);^{6}*D*Lys]

base-labile protecting group:
- Fmoc deprotection with 0.1M HOBt + 2% piperidine + 2% DBU in DMF
- Lys(Mtt) instead of Lys(Dde)

Protoporphyrin IX

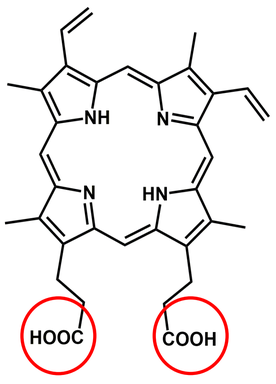
Photodynamic therapy (PDT): combination of a photosensitizer, light and oxygen[4]

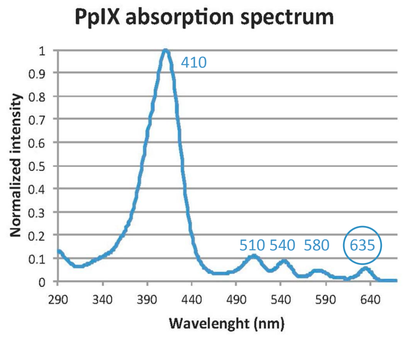
Porphyrin-based photosensitizers are most commonly used due to their photophysical properties[5]



Mechanism of the photodynamic therapy

irradiation with 635 nm light
deeper penetration (~3 mm)



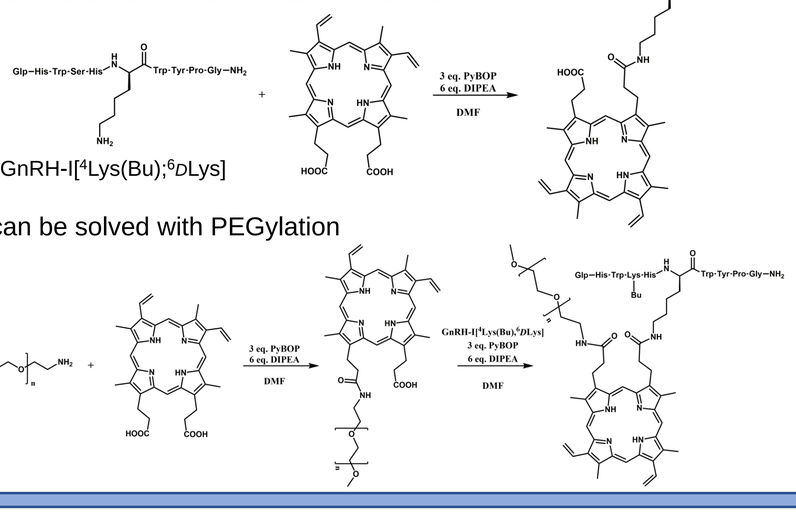


Protoporphyrin IX (PpIX) a second generation photosensitizer

COOH groups suitable for amide bond formation
→ conjugation to targeting moiety

Conjugates

PpIX conjugation *via* amide bond formation in solution



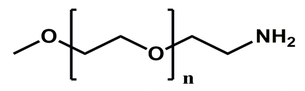
GnRH-I[^{4}Lys(Bu);^{6}*D*Lys]

low solubility can be solved with PEGylation

Conjugate	$MW_{calc.}/MW_{meas.}$ [a]	R_t / min[b]
GnRH-I[^{6}*D*Lys(PpIX)]	1796.9 / 1797.4	24.2
GnRH-I[^{4}Lys(Bu);^{6}*D*Lys(PpIX)]	1908.0 / 1908.5	24.7
PpIX(GnRH-I[^{4}Lys(Bu);^{6}*D*Lys])$_2$	3253.9 / 3255.3	21.8
GnRH-II[^{4}Lys(Bu);^{6}*D*Lys(PpIX)]	1963.3 / 1963.2	24.9
GnRH-III[^{4}Lys(Bu);^{8} Lys(PpIX)]	1929.2 / 1929.3	25.1
PEG-PpIX-(GnRH-I[^{4}Lys(Bu);^{6}*D*Lys])	2889.5# / 2845.6*	24.6
PpIX-PEG	1543.2# / 1499.4*	32.3
PpIX-PEG$_2$	2524.3# / 2349.4*	21.8

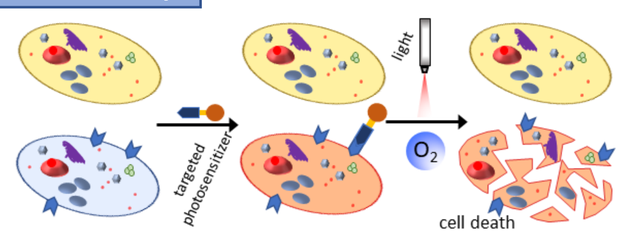
[a] Bruker Daltonics Esquire 3000+ ion trap mass spectrometer
[b] Knauer RP-HPLC, Macherey-Nagel Nucleosil column (250 x 4.6 mm); gradient: 0 min 2%B, 5 min 2%B, 30 min 90%B, 33 min 100%; eluents: 0.1% TFA in H_2O (A), 0.1% TFA in acetonitrile-water 80:20 (B) flow rate: 1 ml/min, detection: λ = 220 nm
calculated with MW_{PEG} mean (999 Da)
* mean $MW_{meas.}$; determined by ESI-MS

PEG



non-toxic, non-immunogenic polymer improves drug solubility and decreases immunogenicity[6]

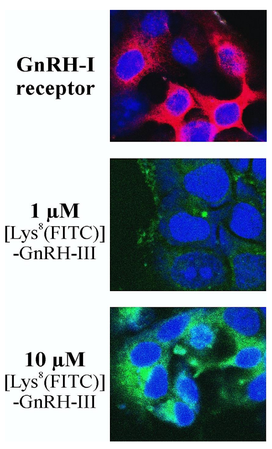
Selectivity



no side-effects (prolonged skin and eye photosensitivity)

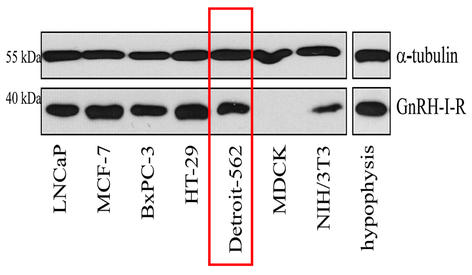
In vitro tests

Detroit-562 human pharyngeal cells



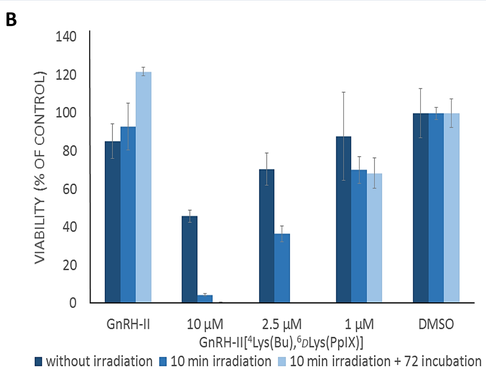
high GnRH-I receptor expression

→ proper cell line for the *in vitro* tests[7]

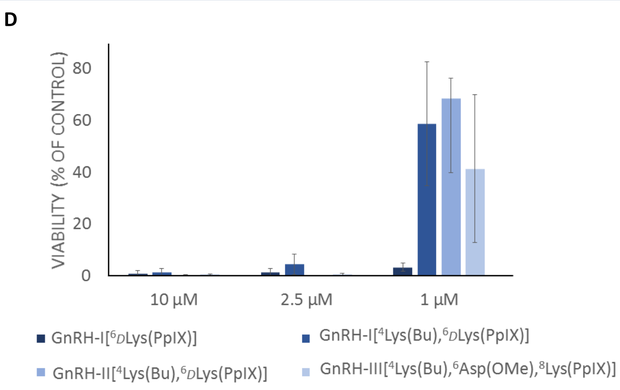


Oriel type 250 W quartz wolfram halogen lamp (QTH lámpa Newport Corporation, CA,USA)

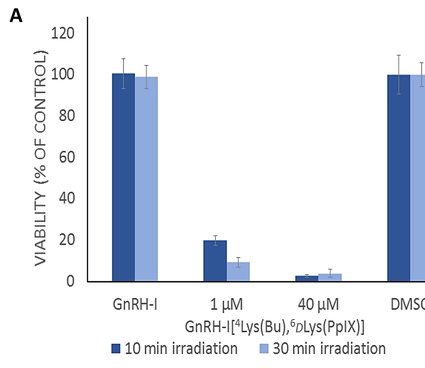
Assay
- 5 h coincubation of the cells with the conjugates
- wash-out
- irradiation (at 635 nm)
- 72 h incubation
- MTT-test



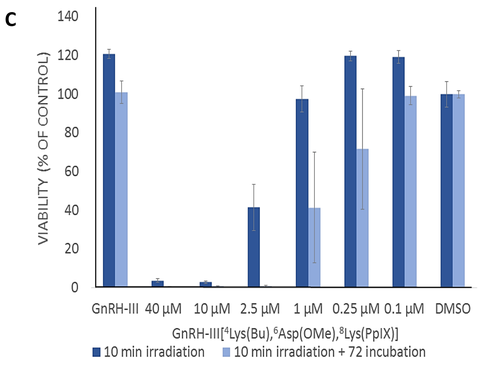
B) optimization of the incubation time
conjugates are not effective without irradiation
toxicity directly after irradiation at in high concentrations



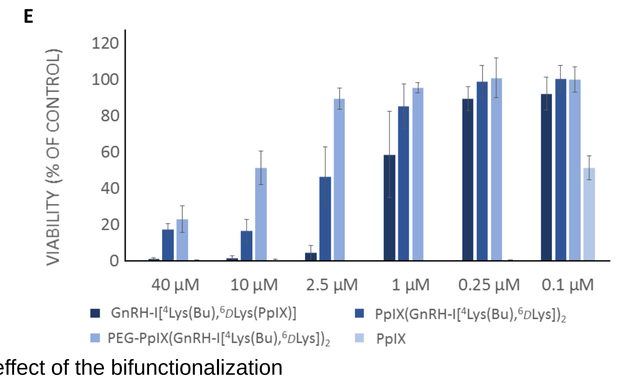
D) effect of the different targeting moieties
the exchange of Ser to Lys(Bu) decreases the efficacy
GnRH-I > GnRH-III[^{4}Lys(Bu)] > GnRH-I[^{4}Lys(Bu)] > GnRH-II[^{4}Lys(Bu)]



A) optimization of the irradiation time
10 min irradiation is almost as effective as 30 min



C) optimization of the concentration
conjugates are effective in low concentrations (~1μM)



E) effect of the bifunctionalization
functionalization of both carboxyl groups leads to higher solubility but lower efficacy

Conclusion

- different GnRH analogs were synthesized
- PpIX was conjugated in solution
- solubility was increased by PEG conjugation
- *in vitro* biological effect was measured by MTT assay
- efficacy is due to the irradiation
- 10 min irradiation is enough
- the conjugates are effective in low concentration (~1μM)
- best conjugate is GnRH-I[^{6}*D*Lys(PpIX)]
- bifunctionalization increases solubility
- bifunctional conjugates have lower efficacy

Acknowledgement

This project has received funding from the National Research, Development and Innovation Office (NKFIH K119552 and NVKP_16-1-2016-0036), Hungary.

References

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