**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 gonad activity and the vertebrate reproduction.

**GnRH-I**
- native ligand of the GnRH receptors, that is synthesized and released in the hypothalamus
- increases receptor binding affinity and stability

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

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

![GnRH](image)

**Synthesis of Fmoc-Rink Amide MBHA resin by SPPS**

- **Glp-His-Trp-Lys-(Dde)-Trp-Lys(Boc)-Leu-Arg(Pbf)-Pro-Gly-Cl**
  - Dde cleavage (2% TFA in DMF)
  - Boc removal (2% TFA in DCM)

- **Glp-His-Trp-Lys-(Bu)-Trp-Lys(Boc)-Leu-Arg(Pbf)-Pro-Gly-Cl**
  - 99% TFA = 2.5% H2O = 2.5% TIS

**Conjugates**

- PpIX conjugation via amide bond formation in solution
- low solubility can be solved with PEGylation

**in vitro tests**

- Detroit-562 human pharyngeal cells
- GnRH-I receptor
- high GnRH-II receptor expression
- proper cell line for the in vitro tests

**Conjugate**

- **Glp-His-Trp-Lys-(Dde)-Trp-Lys(Boc)-Leu-Arg(Pbf)-Pro-Gly-Cl**

**PEG**

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

**Selectivity**

- no side-effects (prolonged skin and eye photosensitivity)

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**Conclusion**

- **different GnRH analogs were synthesized**
- **PpIX was conjugated in solution**
- **in vitro biological effect was measured by MTT assay**
- **efficacy is due to the irradiation**
- **solubility was increased by PEG conjugation**
- **10 min irradiation is enough**
- **bifunctionalization increases solubility**
- **bifunctional conjugates have lower efficacy**
- **best conjugate is GnRH-[(Lys(Bu),7)Lys]**

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**References**