Design and synthesis of Gonadotropin Releasing Hormone (GnRH) peptide analogues conjugated with anthraquinone for selective immunosuppression

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Abstract:

Gonadotropin Releasing Hormone (GnRH or LHRH) is a decapeptide responsible for the control and secretion of the gonadotropin hormones (LH and FSH) and therefore the regulation of the reproductive axis [1]. Altered GnRH peptide analogues have been used for the treatment of fertility problems and hormone-dependent cancer [1, 2]. Drug delivery systems based on GnRH (GnRH conjugates) constitute a more targeted approach for the treatment of hormone-dependent cancer, compared to the effective but with serious side effects conventional chemotherapy protocols. The advantage of the targeted approach stems from the fact that tumor cells express GnRHR receptors in elevated levels compared to healthy cells [3, 4]. The development of a novel and targeted cancer treatment with reduced side effects remains a challenge for the pharmaceutical industry. In this study, a modified anthraquinone molecule was conjugated with GnRH peptide analogues via a disulfide bond [4-7]. The overall aim is the selective binding of the peptide analogue to the GnRH receptors expressed in hormone-dependent cancer cells. Release of the cytotoxic compound *in situ* is expected via reduction of the disulfide bond by the thioredoxin system.

Experimental:

Synthetic Procedures

- Leucoquinizarin was used as a starting material for the synthesis of anthraquinone analogue and was modified for the insertion of a thiol group.
- The GnRH peptide analogues were synthesized step by step by conventional SPPS, following the Fmoc/tBu methodology using Ethyl Indole AM resin, in order to obtain the ethylamide C-terminal. The peptide analogues **A** and **B** were rationally designed and sythesized to simulate the structure of Leuprolide (agonist of GnRH).
- The conjugation between anthraquinone analogue and mutated peptide analogues formatted via a disulfide bond (Figure 1).

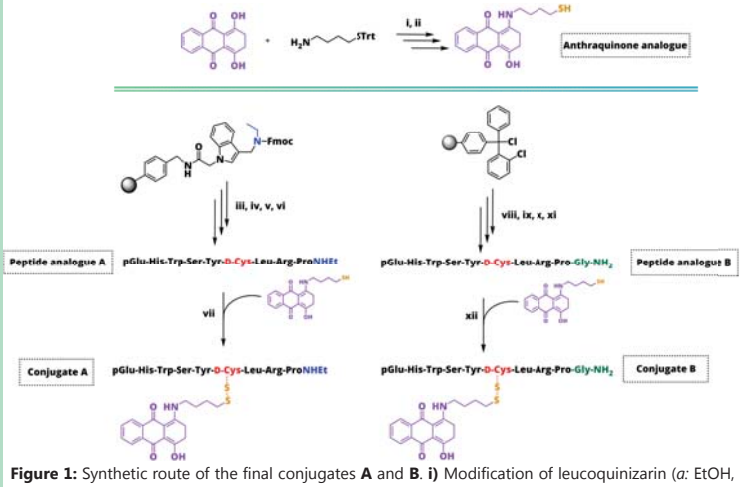


Figure 1: Synthetic route of the final conjugates **A** and **B**. **i)** Modification of leucoquinizarin (*a:* EtOH, reflux; *b:* O2); **ii)** Removal of Trt-protecting group (TFA/DCM/TES, 94:3:3); **iii)** Attachment of the first amino acid on the resin (Fmoc-Pro-OH, HOBt, DIC); **iv)** Synthesis of the desired peptide sequence, (*a:* 25% piperidine/DMF; *b:* Fmoc-AA-OH, HOBt, DIC); **v)** Peptide cleavage from resin (TFA/DCM, 1:1); **vi)** Removal of protecting groups (TFA/DCM/Anisole/H_2O, 90:7:2:1); **vii)** Formation of disulfide bond in DMSO/DIPEA; **vii)** Esterification of Fmoc-Rink Amide linker on the resin (DIPEA); **viii)** Synthesis of the desired peptide sequence, (*a:* 25% piperidine/DMF; *b:* Fmoc-AA-OH, HOBt, DIC); **ix)** Peptide cleavage from resin (DCM/TFE, 7:3); **x)** Removal of protecting groups (TFA/DCM/Anisole/H_2O, 95:2:2:1); **xi)** Formation of disulfide bond in DMSO/DIPEA

Results:

Analytical RP-HPLC and ESI-MS of synthesized conjugates

- The purification and the identification of the analogues were achieved by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) and Electrospray Ionisation Mass Spectrometry (ESI-MS) respectively (Figure 2).

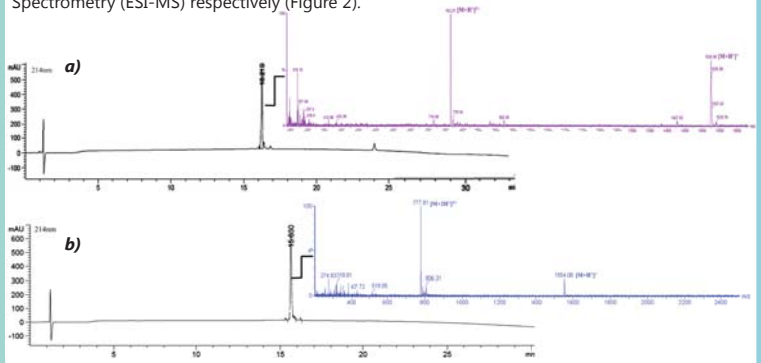
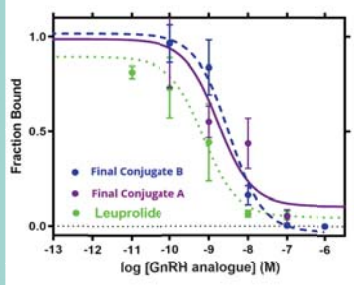


Figure 2: Analytical RP-HPLC and ESI-MS of the synthesized ***a)*** final conjugate A; t_R: 16.22 min; Purity: 94% ; MW=1524.78 and ***b)*** final conjugate B; t_R: 15.63 min; Purity: 97% ; MW=1552.64. Column: Agilent ZORBAX C18; Conditions: 10%(AcN) - 100% (AcN) in 30 min

Binding affinity assay

- The binding affinity of the synthesized conjugates on the human GnRH receptor was determined using competition experiments in HEK 293 cells, while the [^{125}I-*D*-Tyr^6,His^5]GnRH and were used Leuprolide as radioligand and control respectively (Figure 3).



	Leuprolide [DLeu^6,ProNHEt^9]GnRH	Final Conjugate A	Final Conjugate B
$Log IC_{50}$	-9.20	-9.16	-8.70
IC_{50} (nM)	0.64	0.69	2.00

Figure 3: Competition binding isotherms of synthesized conjugate **A** and **B** compared to Leuprolide

Conclusions:

The synthesis of all analogues was achieved successfully without serious synthetic problems. The binding affinity showed that synthesized conjugates **A** and **B** were interacted with GnRH receptor in a dose-dependent manner, and they showed better binding score compared to Leuprolide. Moreover, the synthesized molecules are very promising as drug leads and could be used for further *in vitro* and *in vivo* evaluation against hormone-dependent cancer.

References:

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