GnRH Glycolipids with In Vitro Gonadotropin Hormone Releasing Properties and Direct Antiproliferative Activity on Prostate and Ovarian Cancer Cells
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Purpose
The use of gonadotropin releasing hormone (GnRH) agonists is an important approach for the treatment of gynaecological cancers. The design of an efficient delivery system is a significant point of consideration when developing orally active GnRH analogues. In this study we combined two successful strategies, lipidation and glycosylation, to improve the oral bioavailability of GnRH. Furthermore, Gly6 was substituted by D-Trp6 in four glycolipopeptides to increase the potency.

Methods
Eight GnRH peptides were synthesized by a manual solid-phase protocol (Figure 1). Caco-2 cell homogenate and monolayers were used as models to test the in vitro metabolic stability and permeability, respectively. MTT assay was applied to examine the antiproliferative activity of the constructs (in normal, steroid depleted and estrogen- and dihydrotestosterone (DHT)-reconstituted media) on different tumour cell lines, peripheral blood mononuclear cells (PBMC) and rat pituitary cells. The potency of selected analogues to release FSH was evaluated by ELISA in collagenase-dispersed rat pituitary cells.

Results
All glycolipids showed 4-12 times higher stability and up to 30-fold enhanced permeability compared to GnRH. The highest antiproliferative activity was observed on DU145 and OVCAR-3 (GnRH-receptor positive cells). All constructs, specially the D-Trp6-modified glycolipids, produced significant growth inhibitory effect on DU145 with IC50= 5-25 µM. Five analogues significantly inhibited the proliferation of the OVCAR-3 cells with IC50=45-80 µM. Two of the D-Trp6-modified lipopeptides bearing glucose and lactose induced the release of FSH in rat pituitary cells. All glycolipolipids were shown to be non-toxic on PBMCs and rat pituitary cells. Depletion of the media from steroids significantly reduced the activity of the compounds on DU145 whereas reconstitution with DHT restored the sensitivity of the cells (Figure 2). An opposite effect was observed on OVCAR-3 cells after reconstitution with estrogen.

Conclusion
It was shown that GnRH receptors and steroids play an important role in the growth inhibitory effects of the GnRH glycolipopeptides. Promising lead compound with improved stability and permeability were characterized in vitro to provide new treatment for both hormone dependent and independent cancers. This study also represents another step forward to the oral delivery of GnRH-based therapeutics.