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Synthesis of Modified Endomorphin-1 Peptides

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Objectives: Endomorphin-1 (Endo1, Tyr-Pro-Trp-Phe-NH_2) is a naturally occurring opioid peptide which exhibits high affinity and selectivity for μ-opioid receptors; however, its potent analgesic profile is hampered by very poor metabolic stability and low membrane permeability. To overcome these disadvantages, chemical modifications such as N-terminal modifications of the native peptide by lipidation, glycosylation and/or amino acid substitution of Tyr by non-natural 2,6-dimethyl tyrosine (Dmt) were considered, and a library of Endo1 derivatives was synthesized.[1]
Methods: Amino acid residues were coupled using an Fmoc solid-phase strategy. Dmt was outsourced and protected with an Fmoc group before use. Lipoamino acids bearing 8 and 10 carbon long alkyl chains (C_8Laa and C_{10}Laa) were synthesized from their bromoalkyl precursors and further attached onto the resin using standard coupling reagents. Glycosylated derivatives bore a lactose moiety attached via a succinate linker. All conjugates were purified by RP-HPLC and their mass controlled by ESI-MS.
Results: 6 analogues of Endo-1 were successfully synthesized in overall good yields (range 100-500 mg; 50-95% yield), namely C_8-Endo1, C_{10}-Endo1, Lac-Endo1, and Dmt derivatives C_8-Dmt-Endo1, C_{10}-Dmt-Endo1 and Lac-Dmt-Endo1.
Discussion: Previous *in vitro* evaluations of some of these peptide analogues showed increases in enzymatic stability, membrane permeability and affinity for the μ-opioid receptors.[1] Further research is now being carried out to investigate their *in vivo* pain-modulating activity, side effect profile and mode of action.

[1]Koda Y *et al.*, *Bioorg. Med. Chem.*, **2008**, 16(11), 6286-6296

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Studies on the potential anticancer compounds of Tasmanian *Clematis* species

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Objective. The medicinal plants *Clematis* species (*Ranunculaceae*) are used in folk medicine in China as analgesic, diuretic and anti-inflammatory agents. Recently it was found that some triterpenoid saponins of *Clematis spp.* showed anticancer effects. The purpose of our current study is to assess the anticancer activity of Tasmanian *Clematis* species for their potential as new chemotherapy drug sources.
Methods. In this study, extracts of eleven Tasmanian *Clematis* plants from four species (16μg/mL-8.7mg/mL) were evaluated for dose-dependent cytotoxic effects against the P388 cell line using a colorimetric [3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT)] assay. The effective ones were fractionated on a C18 HPLC column respectively and the chemical constituents were analysed by LC- MS and evaporative light scattering detector to identify the potential compounds and estimate their IC_{50}.
Results. Ten out of these eleven plants had cytotoxic effects on P388 cells. The data demonstrated that the cytotoxic potency of fractions from high to low were *C. microphylla* > *C. aristata* (A) > *C. aristata* (B) > *C. aristata* (C) > *C. gentianoides* > *C. aristata* (D) > *C. aristata* (E) > *C. aristata* (F) > *C. aristata* (G) > *C. vitalba*. The IC 50 of the most effective two (*C. aristata* (A) and *C. microphylla*) were 176μg/mL and 96μg/mL, respectively. The results of their HPLC fractions against the P388 cell line showed that the activity was contributed to 3 fractions containing polar compounds.
Discussion. These findings indicate that Tasmanian *Clematis spp.* contain cytotoxic compounds. These compounds vary between different species and even within the same species growing at different places. The polar compounds will be analysed by ELSD and LC-MS to determine the potential compound structures and estimate their IC_{50}.