

Design, Synthesis and Characterisation of Potent Anti-inflammatory Peptidomimetics

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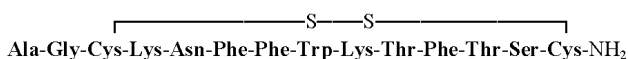
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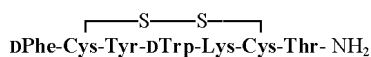
Introduction

Neurogenic inflammation is a critical process in the pathogenesis of numerous inflammatory diseases including bronchial asthma, rheumatoid arthritis, allergic rhinitis, conjunctivitis, eczema, psoriasis and migraine. Neurogenic inflammation is a general term used to describe inflammatory actions induced by local release of some pro-inflammatory substances, e.g. substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A and neurokinin B. These neuropeptides released from stimulated sensory neurons generate redness and warmth (secondary to vasodilatation), swelling (secondary to plasma extravasation) and hypersensitivity (secondary to alterations in the excitability of certain sensory neurons). The intensity of deleterious symptoms could be reduced by decrease of the release of neuropeptides.

Somatostatin is a regulatory peptide widely expressed in the central nervous system and the peripheral tissues in 28 and 14 amino acid-containing forms (Fig. 1. A). Somatostatin regulates several functions of the endocrine system and affects cell proliferation and neurogenic inflammation. Inhibitory effect of somatostatin on inflammation and nociception is well known but the pharmaceutical use of the native peptide and its octapeptide analogues is limited due to their broad spectrum of endocrine effects and short plasma half-life.



A.



B.

Fig. 1. Structure of tetradecapeptide somatostatin (A) and TT-232 (B).

Our research group developed TT-232 (Fig. 1. B), a cyclic heptapeptide analogue of somatostatin that has selective anti-tumour and anti-inflammatory effect without generating other endocrine effects [1-3]. TT-232 is in Clinical Phase II trials. The experiments with non-peptide and peptide analogues of the somatostatin as well as TT-232 verified that three structural units (basic, aromatic and heteroaromatic groups related to L-lysine, L-tyrosine and D-tryptophan) seem to be essential for somatostatin-like biological activity [4,5]. Our aim was to develop small,

non-conventional peptide derivatives expected to have high enzyme resistance, anti-proliferative and anti-inflammatory activity.

Results and Discussion

The common structure of the novel peptide-derivatives is that they have the same, flexible, branched core (X) and three amino acids are situated in equal positions (Table 1). These compounds were synthesised by solution phase technique. After protecting the α -amino and side chain functional groups of the amino acids, they were coupled to the symmetric core in presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. Structure of the synthesised peptidomimetics was proved by mass spectrometry and elemental analysis. A reliable, fast and accurate RP-HPLC method was developed and applied to purify the compounds and characterise their lipophilicity profile. The order of lipophilicity of our peptide-derivatives was determined by both computer calculated logP [6] and experimentally measured retention time. The order of computationally predicted lipophilicity values (logP) and the order of experimentally measured chromatography parameters (t_R) of the compounds fairly correspond with each other (Table 1.).

Table 1. Lipophilicity profile of the compounds

| Code | Compound | t_R^a | $\log P^b$ |
|------|--------------------------------|---------|------------|
| 1 | [Ser] ₃ X | 4,50 | -5,94 |
| 2 | [DTrp] ₃ X | 5,98 | 1,15 |
| 3 | [Boc-Ser] ₃ X | 8,35 | -0,55 |
| 4 | [Lys(Z)] ₃ X | 8,65 | 2,11 |
| 5 | [Z-Glu(OH)] ₃ X | 9,55 | 2,85 |
| 6 | [Lys(2ClZ)] ₃ X | 9,66 | 3,90 |
| 7 | [Tyr(Bzl)] ₃ X | 10,20 | 6,09 |
| 8 | [Boc-Lys(Z)] ₃ X | 11,58 | 7,45 |
| 9 | [Boc-DTrp] ₃ X | 12,87 | 6,70 |
| 10 | [Boc-Lys(2ClZ)] ₃ X | 13,86 | 9,24 |
| 11 | [Z-Glu(OtBu)] ₃ X | 15,25 | 8,33 |
| 12 | [Boc-Tyr(Bzl)] ₃ X | 16,68 | 11,64 |

^a Experimentally measured retention time.

^b Computationally calculated log P.

The biological activity of the peptidomimetics was evaluated by *in vitro* and *in vivo* assays as well. The effect of the compounds on cell proliferation, substance P release and plasma protein extravasation were examined.

***In vitro* cell proliferation assays on two different tumour cell lines.** Human epidermoid carcinoma (A431) and colon cancer (SW480) cells were incubated with different doses of the peptidomimetics. Metabolically active cells were visualised by yellow tetrazolium salt (MTT test). Figure 2 shows the relative activity of the compounds compared to control. Some of the compounds (4, 9, 12) inhibited carcinoma cell proliferation by 30 – 70%.

***In vitro* assay of substance P release.** After exsanguination the tracheae of rats were removed and perfused in an organ bath. Electrical field stimulation was performed to induce the release of substance P from the tissue pieces in the presence and absence of peptidomimetics. Concentration of substance P was determined by specific radioimmunoassay (RIA) and is expressed as a percentage of amount of the neuropeptide released from control tissue (Fig. 3.). Four compounds (4, 8, 9, 11) containing the derivatives of Lys, Glu and Trp strongly inhibited substance P release by 50 – 90%.

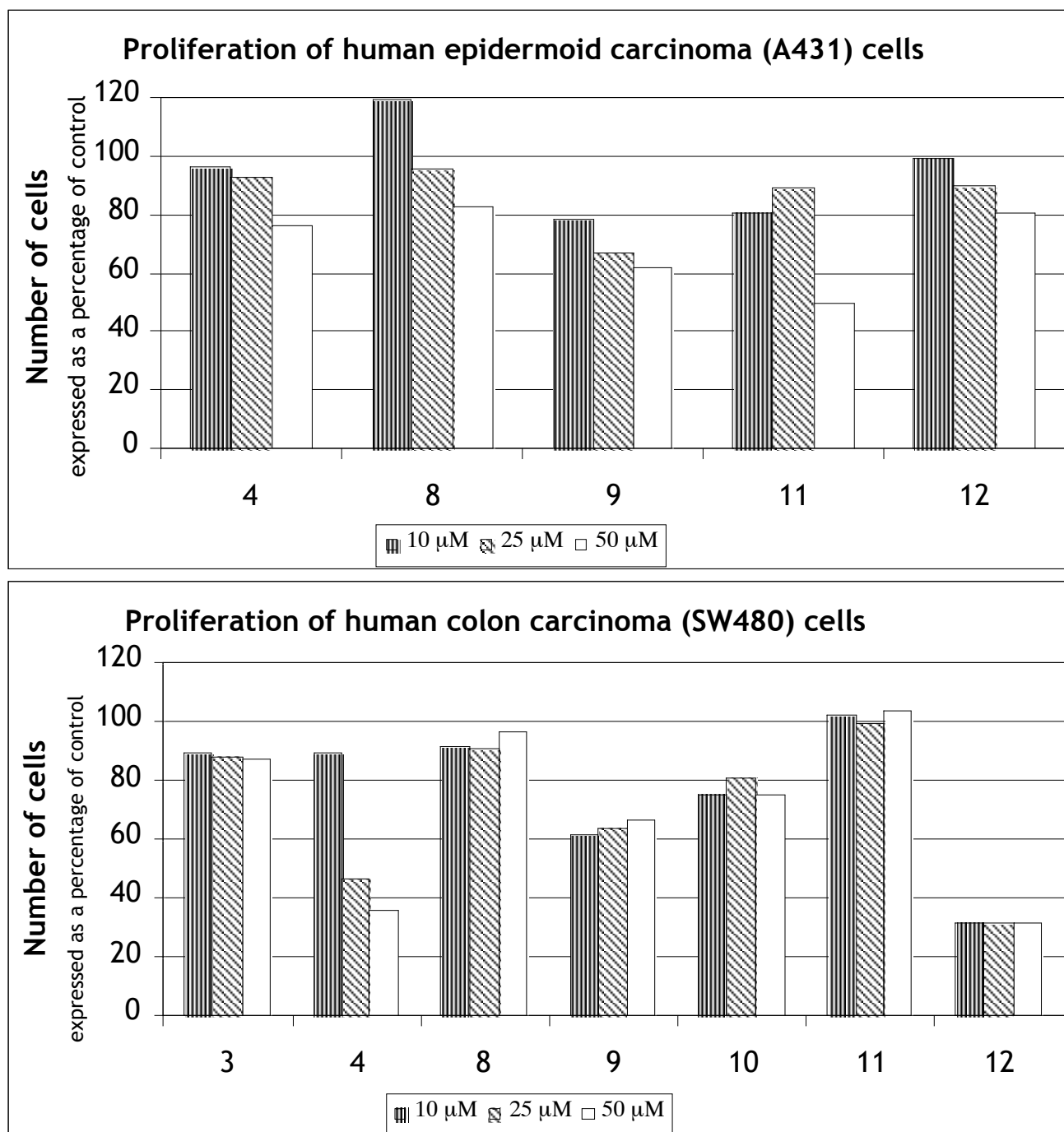


Fig. 2. Effect of the compounds on cell proliferation

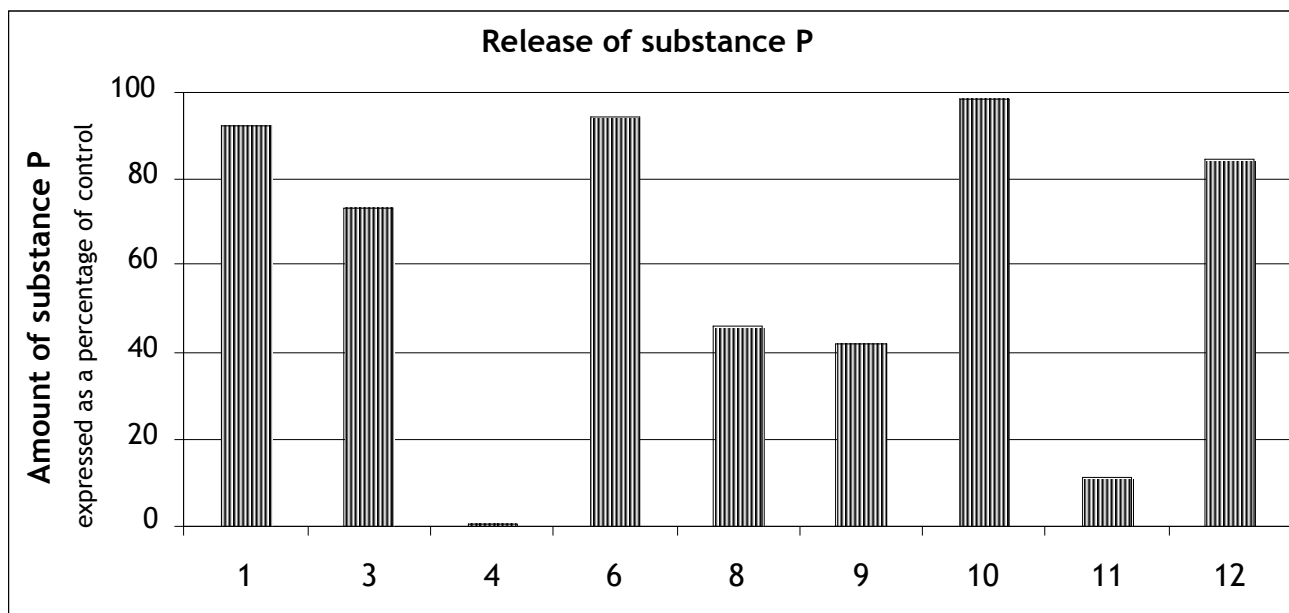


Fig. 3. Effect of the compounds on the release of substance P.

In vivo assay of plasma protein extravasation. Neurogenic inflammation in the skin of the acutely denervated hind leg was evoked by topical application of 1% mustard oil dissolved in paraffin oil. Peptidomimetics, which had significant inhibitory effect on the release of substance P, were administered *per os* in different doses before mustard oil smearing. Extravasation of plasma proteins was measured by the Evans blue leakage method. The amount of the accumulated Evans blue, which quantitatively correlates with the intensity of neurogenic inflammation, is expressed as a percentage of control (Fig. 3.). One compound (8) containing Lys derivative showed strong anti-inflammatory activity when administered orally.

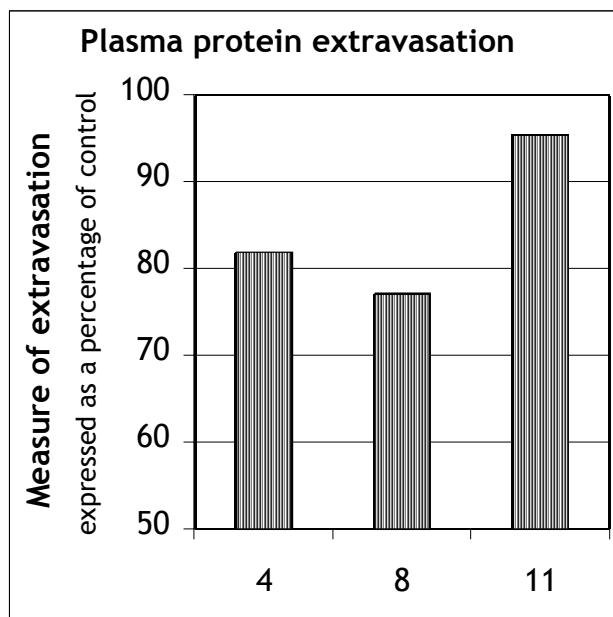


Fig. 4. Effect of the compounds on neurogenic inflammation

Evaluating the structure-activity relationship of the best anti-inflammatory compounds (4, 8, 9, 11) it seems that the presumable essential structural units (basic, aromatic and heteroaromatic) are not required to be in the same molecule. Significant inhibition could be reached when only one or two units are presented in a molecule (heteroaromatic: 9, aromatic: 8, 11, or aromatic and basic: 4).

Acknowledgments

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