

SUPPLEMENTARY MATERIALS

Chemical Synthesis and Structure-Activity Relationship Studies of the Coagulation Factor Xa Inhibitor Tick Anticoagulant Peptide from the Hematophagous Parasite *Ornithodoros moubata*

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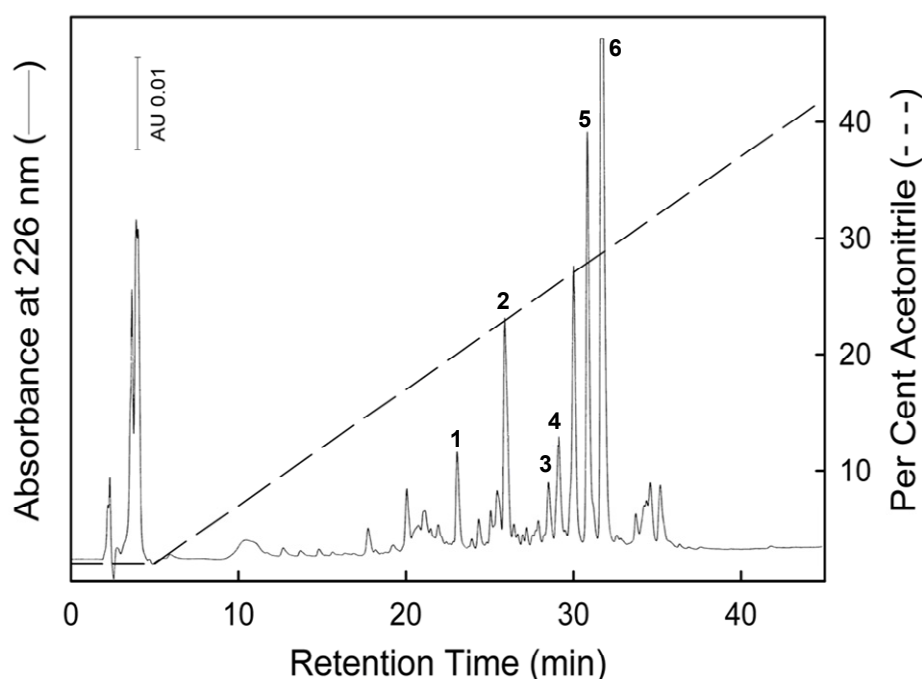
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Table S1. Mass spectrometry data and molar absorptivity values of the synthetic TAP analogues produced in this study.

| TAP | MW (experimental)^a a.m.u | MW (theoretical) a.m.u. | Molar absorptivity^b (ε) at 280nm M⁻¹ ·cm⁻¹ |
|--------------------------------|--|--|--|
| WT | 6978.3 ± 0.2 | 6978.6 | 18,140 |
| Tyr1 <i>p</i> -aminomethyl-Phe | 6979.3 ± 0.5 | 6979.0 | 16,860 |
| Tyr1 <i>p</i> -amino-Phe | 6991.4 ± 0.3 | 6991.6 | 16,860 |
| Tyr1 <i>p</i> -guanido-Phe | 7020.6 ± 0.6 | 7019.7 | 16,860 |
| Tyr1pyridyl-Ala | 6963.8 ± 0.2 | 6963.6 | 16,860 |
| Tyr1β-nafthyl-Ala | 7012.9 ± 0.3 | 7012.6 | 22,240 |
| Arg3 <i>p</i> -aminomethyl-Phe | 6999.2 ± 0.1 | 6999.0 | 18,140 |
| Arg3 <i>p</i> -guanido-Phe | 7026.8 ± 0.4 | 7026.6 | 18,140 |
| Arg3pyridyl-Ala | 6970.9 ± 0.7 | 6970.4 | 18,140 |

^a The values reported refer to the average masses; ^b the values were calculated utilizing molar absorption coefficients of 1280 M⁻¹ cm⁻¹ for Tyr, 5690 M⁻¹ cm⁻¹ for Trp, 120 M⁻¹ cm⁻¹ for Cys and 5380 M⁻¹ cm⁻¹ for βnaftilAla

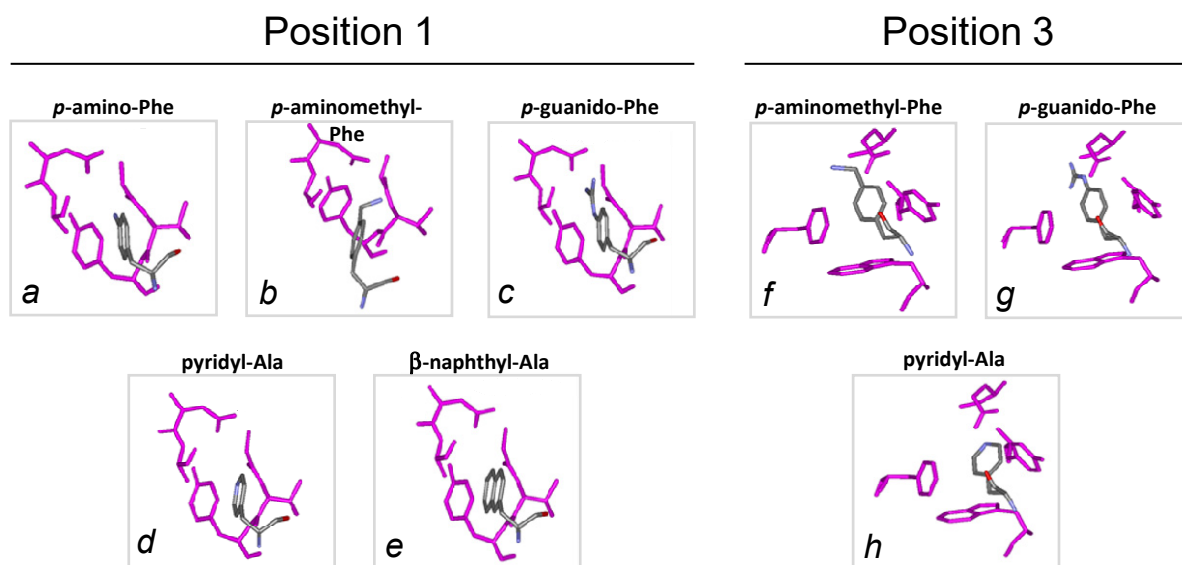


Supplementary Figure S1. Determination of S-S bond topology of N-TAP by peptide mass fingerprint analysis. Purified N-TAP was treated (4 h at 40°C) with trypsin and chymotrypsin, immobilised on Sepharose-4B gel. After filtration, the proteolysis reaction was analysed by LC-MS/MS, and the chemical identity of the material eluted in correspondence of the numbered peaks is reported in the **Supplementary Table S2**.

Table S2. Mass spectrometry data of the peptides obtained by mass fingerprint analysis.

| Peak | Sequence | MW (experimental) ^a a.m.u | MW (theoretical) a.m.u. |
|------|---|--|-------------------------------|
| 1 | ⁵⁷ NACI ⁶⁰ ⁵ CIKP ⁸ | 1032.7 ± 0.3 | 1032.0 |
| 2 | ²⁴ AYF ²⁶ | 400.2 ± 0.1 | 400.2 |
| 3 | ³¹ GGCDSF ³⁶ ⁵³ RDCF ⁵⁶ | 1120.3 ± 0.5 | 1121.6 |
| 4 | ³¹ GGCDSF ³⁶ ⁵⁴ DCF ⁵⁶ | 965.5 ± 0.2 | 964.4 |
| 5 | ⁹ RDWTDECDSNEGGER ²³ ³⁷ WICPEDHTGADY ⁴⁸ | 3027.9 ± 0.6 | 3027.2 |
| 6 | ⁹ RDWTDECDSNEGGER ²³ ³⁷ WICPEDHTGADYY ⁴⁹ | 3189.6 ± 0.3 | 3189.6 |

^a The values reported refer to the average masses.



Supplementary Figure S2. Molecular modelling of TAP analogues interaction with FXa active site. The structure of the synthetic TAP analogues (color coded) in the fXa-bound (magenta) state was modelled using the MOE ver. 15 software, run on the crystallographic structure of TAP-fXa complex (1kig.pdb).