Mini-review

Prospects of sea anemone peptides for pharmacology

Abstract

It has been found that the genes of a number of protein components of sea anemone venoms underwent significant diversification in the course of evolution. The elucidation of the molecular mechanisms of sea anemone peptides interactions with targets let investigators in the last decade actively study the molecular organization and the functioning mechanisms of cytoplasmic membranes, the various types and subtypes of ion channels/receptors involved in the processes of perception, processing, intra- and intercellular signal transduction, both in a body physiological and pathological state. A short characteristic of the structure and functional activity of several classes of sea anemone peptide components, which have pronounced pharmacological potential, is presented in this mini-review.

Keywords: coelenterates, sea anemone, biologically active peptides and polypeptides, protein toxins, molecular organization,

Introduction

The search for natural compounds with pharmacological potential, the study of their diversity consistent patterns of evolutionary development, structure-functional relationships with biological targets, and an effective application in biotechnology and medicine are priority research areas in various fields of life sciences. At the same time, researchers face one of the most urgent tasks. It is the creation of agents highly specific to various targets and having high therapeutic potential aimed at treating socially significant diseases (cancer, neurodegenerative, cardiovascular, etc.) [1]. It is known that marine coelenterates (sea anemones, jellyfishes) producing a huge variety of protein toxins are the most attractive and studied producers of biologically active substances (along with sea snakes and cone mollusks, as well as terrestrial venomous organisms: snakes, arthropods, insects) used for creation promising drugs for pharmacology on their basis [2].

Owing to evolutionary processes of diversification (functionalization, sub- and neofunctionalization), multigene families encoding combinatorial peptide libraries were formed: APETx-like toxins [3-5], pore-forming toxins (actinoporins) [6], Kuntz-type peptides [7], which were evolutionarily selected highly homologous molecules with functional activity aimed at expanding of biological targets in the body of a prey or a potential predator. The search for new natural peptide ligands with high specificity to different biological targets is an urgent task that researchers face [1,2]. The study of the structure-functional relationships of the peptide components of sea anemones is the theoretical basis for searching new targeted actions of pharmacological compounds.

The analysis of the current state of research in this area

To date it has been established that the major components of sea anemone venomous secrets are: neurotoxins (5 kDa) (Figure, **a**, **b**) [8], some toxic and non-toxic APETx-like peptides (4 kDa) (Figure, **c**) [3-5], pore-forming toxins (PFTs) (20 kDa) (Figure, **d**) [6,9], as well as non-toxic Kunitz-type peptides (6 kDa) (Figure, **e**) [7,10-12] and β-defensin-like peptides (4 kDa) (Figure, **f**) [13]. Site-directed mutagenesis, electrophysiological and in silico studies have shown that the selective action of sea anemone protein ligands and their activity are conditioned by the interaction of functionally significant residues with their binding sites on the appropriate biological targets: voltage-gated Nav [8] and Kv [14] ion channels (for **a** and **b**), acid-sensing

(ASICs) channels [3-5] (for **c**) and many other ones [15], cytoplasmic membranes (for **d**) [16], proteases [7,10-12] and/or TRPV1 [17,18] and H-1 histamine [19] receptors (for **e**), pancreatic alpha-amylase [13,20] (for **f**). Using modern biotechnological omics technologies and approaches (genomics, transcriptomics, proteomics as well mass spectrometry), high-throughput NGS and in vitro screening (cell biology and electrophysiology) made it possible to study the structural diversity and functional specificity of toxins produced by sea anemones, as well as the molecular mechanisms of their interaction with various targets [21].

The elucidation of the molecular mechanisms of sea anemone peptides interactions with targets let investigators in the last decade actively study the molecular organization and the functioning mechanisms of cytoplasmic membranes, the various types and subtypes of ion channels/receptors involved in the processes of perception, processing, intra- and intercellular signal transduction, both in a body physiological and pathological state [2]. Thus, it has been reliably established that violation of the functional activity of targets causes various types of channelopathies and pathologies while the blocking or activating effect of sea anemone peptides can have a pharmacological effect on them. So toxic and non-toxic sea anemone venomous peptides, due to their unique and individual properties as well as their possessing large structural diversity and having selectivity/specificity for various targets, are of great interest as a basis for the design of new potential therapeutic agents.

At the moment, a very small amount of effective and safe protein ligands is observed to act selectively on certain ion channels and exhibit a therapeutic effect [22]. From the three structural types of toxins modulating Kv channels, the bifunctional toxins of type 2, kalicludines AsKC 1 – AsKC 3 (*Actinia viridis*, 58–59 aa) are the most studied, they inhibit both trypsin and Kv1.2 and calcium (Ca^{2+})-dependent potassium channels [23]. The presence of pharmacological action was reliably shown only for type 1 toxin, ShK from *Stichodactyla helianthus* (35 amino acid residues) and its recombinant analog, 20kDa-PEG-[Lys16]ShK [23]. Both of them bind selectively to Kv1.1 and Kv1.3 of T-lymphocytes without affecting other cell types, are involved in the regulation of membrane potential and signal transduction, and inhibit the secretion of interleukins IL-17 and IL-4 in monkeys [24]. This ability may be promising in the treatment of autoimmune diseases (multiple sclerosis, rheumatoid arthritis).

Among the fifty representatives of the four structural types (1–4) of neurotoxins (identified to date) (Figure, **a, b**) modulating Nav channels and slowing down the kinetics of their rapid inactivation upon binding to site 3, no pharmacological effect has been found [8]. At the same time, highly homologous sea anemone APETx-like peptides with a similar structural fold, non-toxic peptides Hcr 1b-1 – Hcr 1b-4 (*Heteractis crispa*) (Figure, **c**) are able to inhibit acid-sensing ASIC1a and ASIC3 channels [4,5], exhibiting an anxiolytic effect and sufficiently high anti-inflammatory activity [25]. It has recently been found that APETx-like peptides from sea anemone *Heteractis magnifica* inhibit Nicotinic Acetylcholine Receptors [26]. It has been established that, in addition to homomeric ASIC3, APETx2 (*Anthopleura elegantissima*) (Figure, **c**) inhibits heteromeric ASIC1a/3, ASIC1b/3, and ASIC2b/3 channels, effectively potentiates ASIC1b and ASIC2a ones [3], showing an analgesic effect in the models of acid-induced muscle pain as well as one caused by inflammation.

It has been found that actinoporins StnI from *S. helianthus* and RTX-A (=Hct-A) from *H. crispa* (Figure, **d**), have antitumor activity [16]. Thus, Hct-A is cytotoxic to colon cancer cells (SNU-C4) [21], monocytic leukemia (THP-1), cervical cancer (HeLa), and breast cancer (MDA-MB-231). It prevents epidermal growth factor-induced tumor transformation of mouse JB6P+Cl41 epithelial cells by activating p53-independent apoptosis and inhibiting the activity of oncogenic nuclear factors AP-1 and NF-κB [27]. The ability of RTX-A to interact with membrane integrins of some tumor cells and sea urchin eggs (which lack the lipid receptor of PFTs, sphingomyelin) resulting in the actinoporin antitumor effect and inhibition of egg fertilization processes, is discussed in the review [28]. In recent years the actinoporins StnI, gigantoxin-4 (*Stichodactyla gigantea*), EqtII (*Actinia equina*), and FraC (*Actinia fragacea*) have

been used for creating immunoconjugates with various ligands for targeted cytolytic action on parasitic and tumor cells [29].

The multigene family of *H. crispa* Kunitz-type peptides [7] (Figure, **e**) is a source of a huge variety of representatives which activity determined by certain functionally significant residues is also associated with acting on various targets [10,11,15]. Among the representatives of this family, some belonging to so-called "analgesic cluster" [7] have also been found: APHC1–APHC3 [7,17], HCRG21 [18], HCRG1, HCRG2, HCGS1.10, HCGS1.36, HCGS1.19, HCGS1.20 [7, 10-12] (Figure, **e**),which activity is due to both a trypsin-inhibiting effect [19,20] and inhibition of a vanilloid (TRPV1) receptor [17,18] and some Kvs [15]. Besides, Kunitz-type peptides demonstrate neuroprotective activity in the neurotoxicity model induced by 6-hydroxydopamine [30], as well as peptide HCRG21, blocker of TRPV1, which suppresses TNF-α production and prevents the development of edema and hypersensitivity in acute local inflammation induced by carrageenan [31]. As a result, the peptides exhibit pronounced analgesic and anti-inflammatory activity.

Thus, expanding the basis of pharmacologically active sea anemone peptides will certainly contribute not only to the successful development of new therapeutic agents, but also to a deeper understanding of the mechanisms of functioning of their biological targets.

Conclusion

Thus, large-scale studies of the structure-functional relationships of sea anemone proteinaceous components and data presented in this mini-review on the biological activity of several peptide groups aimed at a wide range of biological targets indicate their high pharmacological potential. This points to the topicality of search for the new producers of biologically active substances among marine coelenterates, which represent one of the richest and most promising natural sources of future drugs.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

References:

1. Prentis P.J., Pavasovic A., Norton R.S. Sea Anemones: Quiet Achievers in the Field of Peptide Toxins // *Toxins* **2018**, *10* (1), 36. https://doi.org/ 10.3390/toxins10010036.
2. Liao Q., Feng, Y., Yang B., Lee S.M.-Y. Cnidarian peptide neurotoxins: A new source of various ion channel modulators or blockers against central nervous systems disease. *Drug Discovery Today* **2019**, *24*, 189–197. https://doi.org/ 10.1016/j.drudis.2018.08.011.
3. Diochot, S.; Baron, A.; Rash, L.D.; Deval, E.; Escoubas, P.; Scarzello, S.; Salinas, M.; Lazdunski, M. A new sea anemone peptide, APETx2, inhibits ASIC3, a major acid-sensitive channel in sensory neurons. *Embo J.* **2004**, *23*, 1516–1525. https://doi.org/ 10.1038/sj.emboj.7600177.
4. Kalina R., Gladkikh I., Dmitrenok P., Chernikov O., Koshelev S., Kvetkina A., Kozlov S., Kozlovskaya E., Monastyrnaya M. New APETx-like peptides from sea anemone *Heteractis crispa* modulate ASIC1a channels. *Peptides* **2018**, *104*, 41–49. https://doi.org/ 10.1016/j.peptides.2018.04.013.
5. Kalina R.S., Koshelev S.G., Zelepuga E.A., Kim N.Y., Kozlov S.A., Kozlovskaya E.P., Monastyrnaya M.M., Gladkikh I.N. APETx-like peptides from the sea anemone *Heteractis*

crispa, diverse in their effect on ASIC1a and ASIC3 ion channels. *Toxins* **2020**, *12*, 266 [1–20]. https://doi.org/ 10.3390/toxins12040266.
6. Leychenko E., Isaeva M., Tkacheva E., Zelepuga E., Kvetkina A., Gusev K., Monastyrnaya M., Kozlovskaya E. Multigene family of pore-forming toxins from sea anemone *Heteractis crispa*. *Mar. Drugs* **2018**, *16*, 183 [1−18]. https://doi.org/ 10.3390/md16060183.
7. Isaeva M.P., Chausova V.E., Zelepuga E.A., Guzev K.V., Tabakmakher V.M., Monastyrnaya M.M., Kozlovskaya E.P. A new multigene superfamily of Kunitz-type protease inhibitors from sea anemone *Heteractis crispa*. *Peptides* **2012**, *34*, 88–97. https://doi.org/ 10.1016/j.peptides.2011.09.022.
8. Monastyrnaya M.M., Kalina R.S. Kozlovskaya E.P. The Sea Anemone Neurotoxins Modulating Sodium Channels: An Insight at Structure and Functional Activity after Four Decades of Investigation. *Toxins* **2023**, *15*, 8 [1−38]. https://doi.org/ 10.3390/toxins15010008.
9. Monastyrnaya M., Leychenko E., Issaeva M., Likhatskaya G., Zelepuga E., Kostina E., Trifonov E., Nurminski E., Kozlovskaya E. Actinoporins from the sea anemones, tropical *Radianthus macrodactylus* and northern *Oulactis orientalis*: Comparative analysis of structure-function relationships. *Toxicon* **2010**, *56*, 1299–1314. https://doi.org/ 10.1016/j.toxicon.2010.07.011.
10. Gladkikh I., Monastyrnaya M., Leychenko E., Zelepuga E., Chausova V., Isaeva M., Anastyuk S., Andreev Y., Peigneur S., Tytgat J., Kozlovskaya E. Atypical reactive center Kunitz-type inhibitor from the sea anemone *Heteractis crispa*. *Mar. Drugs* **2012**, *10*, 1545–1565. https://doi.org/ 10.3390/md10071545.
11. Gladkikh I., Monastyrnaya M., Zelepuga E., Sintsova O., Tabakmakher V., Gnedenko O., Ivanov A., Hua K.-F., Kozlovskaya E. New Kunitz-Type HCRG Polypeptides from the Sea Anemone *Heteractis crispa*. *Mar. Drugs* **2015**, *13*, 6038–6063. https://doi.org/ 10.3390/md13106038.
12. Sintsova O.V., Monastyrnaya M.M., Pislyagin E.A., Menchinskaya E.S., Leychenko E.V., Aminin D.L., Kozlovskaya E.P. Anti-inflammatory activity of the polypeptide of the sea Anemone, *Heteractis crispa*. *Bioorg. Chem*. **2015**, *41*, 590–596. (In Russian).
13. Sintsova O., Gladkikh I., Kalinovskii A., Zelepuga E., Monastyrnaya M., Kim N., Shevchenko L., Peigneur S., Tytgat J., Kozlovskaya E., Leychenko E. Magnificamide, a α-Defensin-Like Peptide from the Mucus of the Sea Anemone *Heteractis magnifica*, Is a Strong Inhibitor of Mammalian α-Amylases. *Mar. Drugs* **2019**, *17*, 542 [1−15]. https://doi.org/ 10.3390/md17100542.
14. Schweitz H., Bruhn T., Guillemare E., Moinier D., Lancelin J.M., Béress L., Lazdunski M. Two different classes of sea anemone toxins for voltage-sensitive K^+ channels. *J. Biol. Chem.* **1995**, *270*, 25121–25126. https://doi.org/ 10.1074/jbc.270.42.25121.
15. Gladkikh I., Peigneur S., Sintsova O., Pinheiro-Junior E.L., Klimovich A., Menshov A., Kalinovsky A., Isaeva M., Monastyrnaya M., Kozlovskaya E., Tytgat J., Leychenko E. Kunitz-type peptides from the sea anemone *Heteractis crispa* demonstrate potassium channel blocking and anti-inflammatory activities. *Biomedicines* **2020**, *8*, 473 [1–17]. https://doi.org/ 10.3390/biomedicines8110473.
16. Fedorov S., Dyshlovoy S., Monastyrnaya M., Shubina L., Leychenko E., Kozlovskaya E., Jin J.O., Kwak J.Y., Bode A.M., Dong Z., Stonik V. The anticancer effects of actinoporin RTX-A from the sea anemone *Heteractis crispa* (=*Radianthus macrodactylus*). *Toxicon* **2010**, *55*, 811–817. https://doi.org/ 10.1016/j.toxicon.2009.11.016.
17. Andreev Y.A., Kozlov S.A., Koshelev S.G., Ivanova E.A., Monastyrnaya M.M., Kozlovskaya E.P., Grishin E.V. Analgesic compound from sea anemone *Heteractis crispa* is the first polypeptide inhibitor of vanilloid receptor 1 (TRPV1). *J. Biol. Chem*. **2008**, *283*, 23914–23921. https://doi.org/ 10.1074/jbc.M800776200.
18. Monastyrnaya M., Peigneur S., Zelepuga E., Sintsova O., Gladkikh I., Leychenko E., Isaeva M., Tytgat J., Kozlovskaya E. Kunitz-type peptide HCRG21 from the sea anemone

Heteractis crispa is a full peptide antagonist of the TRPV1 receptor. *Mar. Drugs* **2016**, *14*, 229 [1–20]. https://doi.org/ 10.3390/md14120229.
19. Sintsova O.V., Chausova V.E., Gladkikh I.N., Isaeva M.P., Tabakmakher V.M., Leychenko E.V., Monastyrnaya M.M., Pislyagin E.A., Menchinskaya E.S., Peigneur S., Tytgat J., Kozlovskaya E.P. New antihistamine Kunitz-type polypeptides of the sea anemones, *Heteractis crispa* and *Stichodactyla mertensii*. *FEBS Journal* **2015**, *282*, suppl. 1, 140–141.
20. Sintsova O., Gladkikh I., Chausova V., Monastyrnaya M., Anastyuk S., Chernikov O., Yurchenko E., Aminin D., Isaeva M., Leychenko E., Kozlovskaya E. Peptide fingerprinting of the sea anemone *Heteractis magnifica* mucus revealed neurotoxins, Kunitz-type proteinase inhibitors and a new β-defensin α-amylase inhibitor. *Journal of Proteomics* **2018**, *173*, 12–21. https://doi.org/ 10.1016/j.jprot.2017.11.019.
21. Robinson S.D., Undheim E.A.B., Ueberheide B., King G.F. Venom peptides as therapeutics: advances, challenges and the future of venom-peptide discovery. *Expert Rev. Proteomics* **2017**, *14*, 931–939. https://doi.org/ 10.1080/14789450.2017.1377613.
22. Pennington M.W., Czerwinski A., Norton R.S. Peptide therapeutics from venom: Current status and potential. *Bioorg. Med. Chem.* **2018**, *26*, 2738–2758. https://doi.org/ 10.1016/j.bmc.2017.09.029.
23. Norton R.S., Pennington M.W., Wulff H. Potassium channel blockade by the sea anemone toxin ShK for the treatment of multiple sclerosis and other autoimmune diseases. *Curr. Med. Chem.* **2004**, *11*, 3041–3052. https://doi.org/ 10.2174/0929867043363947.
24. Beeton C., Pennington M.W., Wulff H., Singh S., Nugent D., Crossley G., Khaytin I., Calabresi P.A., Chen C.-Yin, Gutman G.A., Chandy K.G. Targeting Effector Memory T Cells with a Selective Peptide Inhibitor of Kv1.3 Channels for Therapy of Autoimmune Diseases. *Mol. Pharmacol.* **2005**, *67*, 1369–1381. https://doi.org/ 10.1124/mol.104.008193.
25. Gladkikh I.N., Klimovich A.A., Kalina R.S., Kozhevnikova Y.V., Khasanov T.A., Osmakov D.I., Koshelev S.G., Monastyrnaya M.M., Andreev Y.A., Leychenko E.V., Kozlov S.A. Anxiolytic, analgesic and anti inflammatory effects of peptides Hmg 1b-2 and Hmg 1b-4 from the sea anemone *Heteractis magnifica*. *Toxins* **2023**, *15*, 341[1–20]. https://doi.org/10.3390/toxins15050341.
26. Kalina R.S., Kasheverov I.E., Koshelev S.G., Sintsova O.V., Peigneur S., Lopes Pinheiro-Junior E., Popov R.S., Chausova V.E., Monastyrnaya M.M., Dmitrenok P.S., Isaeva M.P., Tytgat J., Kozlov S.A., Kozlovskaya E.P., Leychenko E.V., Gladkikh I.N. Nicotinic Acetylcholine Receptors Are Novel Targets of APETx-like Toxins from the Sea Anemone *Heteractis magnifica*. *Toxins* **2022**, *14*, 697. https://doi.org/10.3390/toxins14100697.
27. Kvetkina A., Malyarenko O., Pavlenko A., Dyshlovoy S., von Amsberg G., Ermakova S., Leychenko E. Sea Anemone Heteractis crispa Actinoporin Demonstrates In Vitro Anticancer Activities and Prevents HT-29 Colorectal Cancer Cell Migration. *Molecules* **2020**, *25*, 5979 [1–13]. https://doi.org/ 10.3390/molecules25245979.
28. Monastyrnaya M. M., Agafonova I. G., Tabakmakher V. M., Kozlovskaya E. P. The sea anemone pore forming toxins (PFTs): from mechanism of action to perspectives in pharmacology as antitumor agents. *EC Pharmacology and Toxicology* **2023**, *11*, 20–26. https://doi.org/ 10.31080/ecsp.2023.11.00829.
29. Tejuca M., Anderluh G., Dalla Serra M. Sea anemone cytolysins as toxic components of immunotoxins. *Toxicon* **2009**, *54*, 1206–1214. https://doi.org/ 10.1016/j.toxicon.2009.02.025.
30. Sintsova O., Gladkikh I., Monastyrnaya M., Tabakmakher V., Yurchenko E., Menchinskaya E., Pislyagin E., Andreev Y., Kozlov S., Peigneur S., Tytgat J., Aminin D., Kozlovskaya E., Leychenko E. Sea Anemone Kunitz-Type Peptides Demonstrate Neuroprotective Activity in the 6-Hydroxydopamine Induced Neurotoxicity Model. *Biomedicines* **2021**, *9*, 283. https://doi.org/10.3390/biomedicines9030283.
31. Sintsova O., Gladkikh I., Klimovich A., Palikova Y., Palikov V., Styshova O., Monastyrnaya M., Dyachenko I., Kozlov S., Leychenko E. TRPV1 Blocker HCRG21 Suppresses TNF-α Production and Prevents the Development of Edema and Hypersensitivity in

Carrageenan Induced Acute Local Inflammation. *Biomedicines* **2021**, *9*, 716. https://doi.org/10.3390/biomedicines9070716.

a

```
RTX-I          ASCKCDDDGPDVRSATFTGTVDFAYCNAGWEKCLAVYTPVASCCRKKK
RTX-II         GTCKCDDDGPDVRTATFTGSTEFANCNESWEKCLAVYTPVASCCRKKK
RTX-III        GNCKCDDEGPYVRTAPLTGYVDLGYCNEGWEKCASYYSPIAECCRKKK
RTX-IV         GNCKCDDEGPNVRTAPLTGYVDLGYCNEGWDKCASYYSPIAECCRKKK
RTX-V          GNCKCDDEGPNVRTAPLTGYVDLGYCNEGWEKCASYYSPIAECCRKK-
RTX-VI         GNCKCDDEGPYV TAPLTGYVDLGYCNEGWEKCASYYSPIAECCRKKK
δ-SHTX-Hcr1f   ASCKCDDDGPDVRSATFTGTVDFWNCNEGWEKCTAVYTPVASCCRKKK
Rp-II          ASCKCDDDGPDVRSATFTGTVDFWNCNEGWEKCTAVYTPVASCCRKKK
SHTX-IV        AACKCDDDGPDIRSATLTGTVDFWNCNEGWEKCTAVYTAVASCCRKKK
Sh1            AACKCDDEGPDIRTAPLTGTVDLGSCNAGWEKCASYYTIIADCCRKKK
```

b

```
ATX-II   -GVPCLCDSDGPSVRGNTLSGIIWLA--GCPSGWHNCKKHGPTIGWCCKQ
ApA      -GVSCLCDSDGPSVRGNTLSGTLWLYPSGCPSGWHNCKAHGPTIGWCCKQ
ApB      -GVPCLCDSDGPRPRGNTLSGILWFYPSGCPSGWHNCKAHGPNIGWCCKK
```

c

```
               1               17                      41
Hcr 1b-1       GTPCKCHGYIGVYWFMLAGCPNGYGYNLSCPYFLGICCVDR-
Hcr 1b-2       GTPCKCHGYIGVYWFMLAGCPNGYGYNLSCPYFLGICCVKK-
Hcr 1b-3       GTPCKCHGYIGVYWFMLAGCPDGYGYNLSCPYFLGICCVKK-
Hcr 1b-4       GTPCDCYGYTGVYWFMLSRCPSGYGYNLSCHYFMGICCVKR-
APETx1         GTTCYCGKTIGIYWFGTKTCPSNRGYTGSCGYFLGICCYPVD
APETx2         GTACSCGNSKGIYWFYRPSCPTDRGYTGSCRYFLGTCCTPAD
```

d

```
RTX-A    --ALAGAIIAGASLTFQVLDKVLAELGQVSRKIAIGIDNESGGSWTAMNAYFRSGT
           TDVILPEFVPNQKALLYSGRKNRGPDTTGAVGALAYYMSNGNTLGVMFSVPFDY
           NNWWDVKVYSGKRRADQAMYEDLYYSNPYRGDNGWHQKNLGYGLKMKGILYSMT
           SAGEAIMEIRISR      Q

StII    ---ALAGTEELGKVSRKIAVGIDNESGGTWTALNAYFRSGT
           TDVILPEFVPNTKALLYSGRKDTGPVATGAVAAFAYYMSSGNTLGVMFSVPFDYNWY
           SNWWDVKIYSGKRRADQAMYEDLYYGNPYRGDNGWHEKNLGYGLRMKGIMTSAG
              EAKMQIKISR
```

e

```
                           P1
APHC1      GSICLEPKVVGPCTAYFRRFYFDSETGKCTVFIYGGCEGNGNNFETLRACRAICRA
APHC2      GSICLEPKVVGPCTAYFRRFYFDSETGKCTPFIYGGCEGNGNNFETLRACRAICRA
APHC3      GSICLEPKVVGPCTAYFPRFYFNSETGKCTPFIYGGCEGNGNNFETLRACRGICRA
InhVJ      GSICLEPKVVGPCTAYFPRFYFDSETGKCTPFIYGGCEGNGNNFETLRACRAICRA
HCGS1.10   GSICLEPKVVGPCTAYLRRFYFDSETGKCTPFIHGGCEGNGNNFETLRACRAICRA
HCGS1.36   GSICLEPKVVGPCTAYFRRFYYDSETGKCTPFIYGGCEGNGNNFETLRACRAICRA
HCGS1.19   GSICLEPKKVGRCRGSFRRFYFDSETGKCTPFIYGGCGGNGNNFETLHACRAICRA
HCGS1.20   GSICLEPKVVGPCKARIRRFYFDSETGKCTPFIYGGCEGNGNNFETLRACRAICRA
HCRG1      RGICSEPKVVGPCKAGLRRFYYDSETGECLPFIYGGCKGNKNNFETLHACRGICRA
HCRG2      RGICLEPKVVGPCKARIRRFYYDSETGKCLPFIYGGCGGNGNNFETLHACRGICRA
HCRG21     RGICSEPKVVGPCTAYFRRFYFDSETGKCTPFIYGGCEGNGNNFETLRACRAICRA
BPTI      RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQTFVYGGCRAKRNNFKSAEDCMRTCGGA
                  *******                   *******
               reactive site          weak interactions
                                            site
```

f

Magnificamide	SEGTS**C**YIYHGVYGI**C**KAK**C**AEDMKAMAGMGV**C**EGDL**CC**YKTPW
Helianthamide	ESGNS**C**YIYHGVSGI**C**KAS**C**AEDEKAMAGMGV**C**EGHL**CC**YKTPW

Figure.1. Multiple sequence alignments of representatives of sea anemone peptides and polypeptides belonging to the different peptide groups, such as neurotoxins of type 2 (**a**): RTX-I, RTX-II, RTX-III, RTX-IV, RTX-V, RTX-VI, δ-SHTX-Hcr1f from *Heteractis crispa*, Rp-II from *Radianthus paumotensis*, SHTX-IV from *Stichodactyla haddoni*, Sh1 from *Stichodactyla helianthus*; neurotoxins of type 1 (**b**): ATX-II from *Anemonia sulcata*, ApA, ApB from *Anthopleura xanthogrammica* [8]; APETx-like peptides **(c):** Hcr 1b-1 – Hcr 1b-4 [4,5] from *H. crispa* and APETx1, APETx2 from *A. elegantissima* [3]; pore-forming toxins (**d):** RTX-A from *H. crispa* [6] and StII from *S. helianthus* [9]; Kunitz-type peptides (**e):** APHC1 – APHC3 [7], InhVJ [10], HCRG1, HCRG2 [7,11], , HCGS1.10, HCGS1.36, HCGS1.19, HCGS1.20 from *H. crispa* [7,12], HCRG21 [7]; β-defensin-like peptides (**f**): magnificamide from *H. magnifica* and helianthamide from *S. helianthus* [13]. Highly homologous residues and/or sequence fragments are indicated in color. Identical amino acid residues are shown on a gray (**a**, **b**, **c**) and black (**d**) background, point substitutions of residues in the sequences are shown on white; Arg13 residue, functionally significant for the binding of neurotoxins to Na_Vs (**a**, **b**), as well as RGD tripeptide binding of PFTs to integrins (**d**) are highlighted in red. The P1 residue is shown (**e**): residues Lys, Arg, Thr, which determine the interaction of Kunitz peptides with targets, proteases and/or TRPV1 receptor, are highlighted in red. The straight lines at the top of (**a**) show C1-C5, C2-C4, C3-C6 disulfide bridges between cysteine residues (shown in bold). All alignments are made with the help program Vector NTI.