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ABSTRACT

Cnidarians are soft bodied animals possessing complex venom systems which have evolved to allow for the capture of arthropod and vertebrate prey, as well as to defend themselves against such predators. The effects of these venoms on humans, as a result of envenomation, has been studied for many decades, whereas the possibility of using these proteins to fight human disease is in its infancy. Drug discovery utilisation of Cnidarian venoms has been hampered by availability of animals and suitable extraction techniques that allow for study of such protein toxins. Studies of toxins that have been suitably purified for drug discovery have, by in large, only investigated target engagement and negated to investigate other drug like properties such as absorption, dispersion, metabolism, and excretion (ADME). This chapter will review the sourcing of Cnidaria for drug discovery, extraction of venom components, actions of venoms on drug relevant targets and their suitability as drug like molecules.

KEYWORDS

Cnidarian, venom, toxins, ADME, Drug discovery, Drug targets, extraction techniques

INTRODUCTION

Venom has been recruited across the Animal Kingdom as a means of facilitating the acquisition of prey, defence against predators, intra- and interspecific resource competition, and reproduction, evolving convergently over 101 times throughout the evolutionary timeline [1-6]. As a result, the planet contains an immense array of venomous taxa across a diverse range of lineages, and of these lineages the Phylum Cnidaria is thought to be one of the oldest venomous taxa still extant today, with origins dating as far back as 750mya and fossil records showing a major radiation around 550mya [7,8]. Despite being one of the oldest known groups of venomous organisms, the current understanding of Cnidarian toxins falls much shorter than that of other venomous animals and this is due largely to the difficulties associated with obtaining venom samples. It can be inferred from the currently available literature, that the range of Cnidaria used in the study of their toxins is focused on a relatively small pool of species when equated to the sheer diversity of organisms within the Phylum, with the majority of samples being obtained from a number of Jellyfish species (Subphylum Medusozoa) [8-12] and Sea Anemone species (Class Actiniaria) [11,13,14], whilst Coral species appear to have fostered significantly less research attention. Figure 1 shows the annual publication rate for each of the major Cnidarian lineages between 1975 and 2019 [15]. The earliest studies of the Cnidarian venom apparatus were conducted in the mid to late 1800's and

focused heavily on the structure, firing mechanisms, and differentiation of each variety of nematocyst, and it was these studies that provided the fundamental knowledge required to begin exploring ways in which venom samples could be obtained from these microscopic structures [16-20]. Despite the issues related to their extraction in the 'earlier years', Cnidarian toxins were beginning to be utilised for research purposes over 100 years ago and were vital in the Nobel Prize winning research by Charles Richet and Paul Portier which led to the discovery of anaphylaxis [20-23]. The mid-20th Century saw an increased focus towards attempting to reliably obtain venom from Cnidaria in order to develop our understanding of the toxins which they possess, and in turn enhance our ability to explore the activities these toxins exhibit and the therapeutic potential which may be present within.

CNIDARIANS IN DRUG DISCOVERY

Sourcing Cnidaria for Drug Discovery

Cnidaria are thought to be ubiquitous and are present throughout all marine biomes, inhabiting a range of environments; from temperate and tropical shallows to the depths of the Hadal Zone [24,25,26]. As a result, sourcing Cnidaria for drug discovery purposes may not always be an issue, should the target species be a native to the research institution(s). However, this not always the case and instead obtaining specific species may pose some logistical challenges. Travelling for bioprospecting purposes is a viable option and, using SCUBA and Deep-Sea Exploratory systems, even species previously considered relatively inaccessible are now potentially available for sampling [27]. Although there have been improvements in the practical ability to access Cnidarian venoms *in situ*, there are certain caveats which may limit such bioprospecting expeditions such as protection from CITES, national regulatory bodies such as the Great Barrier Reef Marine Park Authority, and the Nagoya Protocol, which may require permit applications in order to undertake sampling [28,29]. The Nagoya Protocol is correctly referred to as the Nagoya Protocol on access and benefit sharing as an addition to the Convention on Biological Diversity [28]. Another option for sourcing Cnidaria, which circumvents some of the legislative constraints surrounding the bioprospecting of Cnidaria *in situ*, is aquaculture. The ability to culture Cnidaria *ex situ* is a relatively well-developed field, with a large focus on cultivation for the ever-growing hobbyist market and a drive towards captive reproduction for conservation in public aquaria and academia [30,31,32]. Although successful Cnidarian husbandry requires adherence a broad range of important parameters such as, water quality, water flow, lighting intensity, temperature, proximity to other species (particularly with certain species of Scleractinia), and diet, modern aquaculture equipment has advanced to a point in which the adherence to and monitoring of the majority of these parameters can be fully automated, increasing the success of aquarists [31,33-36]. The developments in modern husbandry practices and aquarist knowledge has produced advances in the longevity, health, and reproduction of captive Cnidaria. In turn this has created a situation in which Cnidaria cultivation within the laboratory is not only more feasible than ever but has also led to the identification of several Cnidarian species as new model organisms for research purposes. [31,37-40].

Extraction of Cnidarian Venom

There have been a variety of methods developed to extract venom samples from Cnidarian tissues throughout the years, with initial attempts utilising electrical stimulation of nematocysts from the

dissected tentacles of *Chironex fleckeri* which had been placed on to human amnion [12,41,42,43]. Potential contamination of the samples from amniotic proteins led to the formulation of different approaches of obtaining venom, the majority of these relied on several methods of tissue disruption followed by centrifugation or lyophilisation to obtain crude venom [12,43,44,45]. Despite the large variety of techniques in which toxins have been obtained from tissues, the vast majority of methods have yielded results that are of uncertain reliability, have encountered issues with protein denaturation related to the extraction method, have led to the subsequent contamination of sample from tissue proteins, or require laborious methodologies to filter samples [10,11,12,43-47].

2015 saw a major leap towards reliably obtaining clean samples through the development of a technique which employed chemical extraction of venom. This method, which relied on the use of ethanol to initiate the nematocyst firing, proved to yield samples that displayed no contamination from non-target tissues and proteins [10,12]. The future direction of Cnidarian venom research may have turned a corner as a resulting factor of this development. For instance; the improved reliability of samples and the reduction in contamination has, when compared to previous means of extraction, potentially increased the accuracy of venom assays and led to a larger range of venom components which are able to be identified [12].

The development of a means to chemically elicit the firing of nematocysts has greatly improved the potential for *in situ* sampling of Cnidarian toxins as the method has proven to yield rapid expulsion of nematocysts and toxins [12,35]. The use of this method, however, has not gone without challenge, recent research [46] has highlighted a potential problem with the viability of cardiotoxic components of venom samples which have been procured using ethanol extraction. With the results suggesting that there is a level of degradation occurring with the venom proteins when extracted using ethanol, when compared to venom extracted in sea water using beads to cause disruption of the nematocysts. The findings of this research have further emphasised a greater need to thoroughly explore all aspects of chemical extraction in order to fully assess the viability as a practical solution to obtain Cnidarian venom samples. There are potential benefits to the use of chemical extraction which are unique to this method and as such would not be feasible with other available extraction techniques. One such example of this is the almost instant delivery of venom from this method, which has presented a niche in which there is an ability to combine the chemical extraction technique with a proprietary device that is able to pass a flow of alcohol over the tissues whilst simultaneously drawing up the resulting mixture of ethanol, toxins and surrounding water. The development of such a device could potentially allow for the *in situ* sampling of a vast array of Cnidarian species across the entire Phylum which could, in turn, rapidly accelerate our understanding of Cnidarian toxins as a result.

Cnidarian Venom Composition and Activity

Owing to the technicalities pertaining to extraction and preparation of Cnidarian venoms being present as a limiting factor, our knowledge of their toxin composition is an area in which is still not widely understood, despite there being constant advances in availability of screening techniques [12,14,48-51]. With the development of extraction and purification techniques for Cnidarian venoms leading to an ability to research toxin composition from a greater number of species, we are slowly beginning to find a much more diverse array of compounds than were previously known. An example can be seen in small cysteine-rich peptides (SCRiPs) that were initially thought to be genes involved in calcification and reef formation in Scleractinia, however

several homologous peptides were also identified in Actiniaria. When isolated from *Acropora millepora* these SCRiPs were found to exhibit acute neurotoxicity in *Danio rerio* fry, and as a result are thought to be the first known example of neurotoxic compounds within Scleractinian venom [52-54]. Table 1 highlights the current state of our understanding of Cnidarian toxins, including the taxa in which the toxins have been currently observed; the toxin families; and the activities exhibited by these toxins.

Nomenclature of Cnidarian Toxins

Given the complex nature of many venoms, it is reasonable to suggest that a unified method of nomenclature would benefit the field of venom research. One such suggestion came in the form of the proposed rationalisation of peptide and protein toxin nomenclature by King *et al* in 2008 [55]. Through the use of this format, a researcher could theoretically observe a toxin unknown to them and, with an understanding of the rationalised format, extrapolate a series of information about the toxin and the animal from which it originated. This format works through using a prefixed Greek letter which acts as a descriptor for a specific activity, after this is the family name of the animal in which the toxin originates, this is followed by a three letter sequence which denotes the genus and species of the animal, following this is a number to differentiate between pharmacologically comparable toxins, and finally a letter is assigned to indicate the presence of isoforms [55,56]. For example, the Greek lower-case letter kappa (κ) denotes that there is an inhibitory activity on the Voltage-Gated Potassium (K_v) Channels or the lower-case Greek letter delta (δ) signifies that the toxin causes a delay in the inactivation of the Voltage-Gated Sodium (Na_v).

Using *Anemonia viridis/sulcata* as an example species, the toxins formally named BDS-1 and BDS-2 do not present any real information relating to the species or the fact that the toxins have a K_v modulating activity. However, when presented in the rationalised format; k-Actitoxin-Avd4a and κ-Actitoxin-Avd4b respectively, it shows that these toxins act on K_v channels, they are toxins originating from the Actiniidae family, they are from the species *Anemonia viridis*, they are one of at least 4 unrelated toxins which share a similar pharmacology, and there are a series of isoforms of κ-Actitoxin-Avd4a. Similarly, using the toxins ATX-I, ATX-II, ATX-III, and Av7 as another example series. These toxins all act on the Na_v channels and are all found in *Anemonia viridis* once again. When rationalised they would become; δ-AITX-Avd1a and δ-Actitoxin-Avd1ab for ATX-I, δ-Actitoxin-Avd1c for ATX-II, δ-Actitoxin-Avd2a for ATX-III, and δ-Actitoxin-Avd2b for Av7. This shows that the toxin formally known as ATX-I has three isoforms, one of which was formally thought to be a separate toxin in ATX-II, and ATX-III is a separate toxin of which Av7 is an isoform [56].

When a toxin is presented in this format it is clear that, when compared to the currently utilised non-standardised toxin nomenclature, there is often a significant lack of clear information about a specific toxin which can be obtained using current means of naming toxins. Presenting toxins in a rationalised format is, at present, an under-utilised practice. As a result, toxin nomenclature as a whole is in a state in which a large-scale review would be required to implement the format proposed by King *et al* in 2008 [55]. The current state of toxin nomenclature undoubtedly requires some reclassification in a rationalised format and this would ideally serve to expand the work which Oliveira *et al* undertook to reclassify Sea Anemone toxins in 2012 [56]. During this chapter the nomenclature has been used that is as published by the relevant authors.

Table 1. Current Understanding of the Venom Composition of Cnidaria. Taken from [15].

Cnidarian Class	Toxin Family	Activity	Molecular Weight (kDa)	References
Anthozoa, Scyphozoa[1]	NaTxs	Neurotoxicity, Cardiotoxicity, Insecticidal	3-8kDa	[8,53,57,58]
Anthozoa, Scyphozoa[1]	KTxs	Neurotoxicity, Cardiotoxicity, Analgesia, Hypotensive, Immunosuppressive, Antimicrobial	3-5.8kDa	[8,14,53,58,59,60]
Anthozoa	ASIC Inhibitors	Analgesia, Neurotoxicity	3-4.7kDa	[8,53,59,61]
Anthozoa	TRPV1 Inhibitors	Analgesia, Serine Protease Inhibitor	3kDa	[8,53,59]
Anthozoa, Scyphozoa	Three-Finger Toxin-Like Proteins[2]	Neurotoxicity	2-14.5kDa[3]	[62-64]
Anthozoa, Scyphozoa[1]	Kunitz Peptides	Neurotoxicity, Serine Protease Inhibitor, Paralytic	6-7kDa	[8,53,58,59,62,65]
Anthozoa	SCRiPs	Paralytic	4.3-8kDa	[8,22,53,60]
Anthozoa, Hydrozoa	Actinoporins	Cytolytic, Haemolytic, Cardiotoxicity, Myotoxicity	20-22kDa	[8,14,53,66-68]

[1] The presence of these toxins in Scyphozoa are a relatively new discovery and, although included in this table, there is a definite need for further study to assess their roles within the venom.
[2] Currently only observed in a limited number of species.
[3] Information gathered through UniProtKB search (https://www.uniprot.org/uniprot/?query=three-finger+toxin&sort=mass&desc=no).

Table 1. (Cont.) Current Understanding of the Venom Composition of Cnidaria

Cnidarian Class	Toxin Family	Activity	Molecular Weight (kDa)	References
Anthozoa, Scyphozoa	C-Type Lectins	Pro/Anticoagulant, Disruption of Platelet Activation	14-30kDa	[58,62,69-72]
Scyphozoa[1]	Scyphozoan Pore-Forming Toxins (PFTs)	Haemolytic, Cytolytic	36-55kDa	[58,72-73]
Cubozoa	Jellyfish Toxins	Haemolytic, Cardiotoxicity, Cytolytic, Myotoxicity, Inflammatory	42-52kDa	[8,67-68]
Anthozoa, Hydrozoa	Hydralysins	Cytolytic, Paralytic, Haemolytic, Aids Digestion of Prey	27-31kDa	[8,50,75]
Anthozoa, Scyphozoa, Hydrozoa	Membrane Attack Complex-Perforin (MACPF) and MACPF Homologues	Cytolytic, Haemolytic, Cytotoxicity	~3&60kDa	[8,50,62,75-76]
Anthozoa, Scyphozoa, Cubozoa, Hydrozoa	Phospholipase A_2 (PLA_2)	Cytolytic, Haemolytic	13-95kDa	[8,58,62,64,66,77]
Anthozoa, Scyphozoa, Cubozoa	Metalloproteases	Cytolytic, Hemolytic, Cytotoxicity	17-130kDa	[8,58,62,68,71,72]
Scyphozoa	Hyaluronidase[2]	Toxin Transportation via Breakdown of Extracellular Matrix	55&95kDa	[58,78]
Scyphozoa	L-Amino Acid Oxidases (LAAOs)	Cytotoxicity, Haemolytic, Platelet Aggregator, Haemorrhagic, Cell Apoptosis	120-150kDa	[58,79,80]

Table 1. (Cont.) Current Understanding of the Venom Composition of Cnidaria

Cnidarian Class	Toxin Family	Activity	Molecular Weight (kDa)	References
Anthozoa, Hydrozoa	5-Hydroxytriptamine	Vasodilatory, Nociceptor Agonist	176.22	[8,68,74]
Anthozoa	Histamine	Vasodilatory, Nociceptor Agonist	111.15	[8,68]
Anthozoa	Caissarone	Vasodilatory, Adenosine Receptor Antagonist	193.21	[8,22,50,66]
Anthozoa	Bunodosine	Vasodilatory, Analgesia	391.22	[8,50,66]

THERAPEUTIC POTENTIAL OF CNIDARIAN TOXINS

There is much evidence that venom components can be developed into new therapeutics as there are already a number of drugs on the market that have been developed from venoms. Most of these so far have been utilised from reptile venom. Capoten® (Captopril) which treats hypotension was the first drug to be developed from a component of venom and this venom was from the Brazilian or Arrowhead Pit Viper *Bothrops jararaca* [81]. A drug that treats angina Aggrastat® (Tirofiban) was derived from the venom of the Saw-Scale Viper *Echis carinatus* [81]. Integrilin® (Eptifibatide) is another angina drug from the South-Eastern Pygmy Rattlesnake *Sistrurus miliarius barbouri* [82]. Defibrase® (Batroxobin) developed to treat thrombosis was produced from venom components discovered in both the Common Lancehead *Bothrops atrox* and the Brazilian Lancehead *Bothrops moojeni* [83]. Byetta® (Exenatide) from the Gila Monster *Heloderma suspectum* was developed to treat type 2 diabetes [84].

There are currently two drugs that originate from a marine animal, Prialt® (Ziconotide) is one of these. This drug has been developed to treat chronic pain and the active component is a peptide called omega-conotoxin which has been developed from the venom of the Magical Cone Snail *Conus magus* [85]. This peptide acts on voltage sensitive calcium channels by blocking them. The other is from a Cnidarian and is called Dalazatide and was originally derived from the Sea Anemone *Stichodactyla helianthus* [59]. This is currently in clinical trials and you can read more about this in the section below on autoimmune disease.

Although there are currently no clinically approved drugs available which are derived from the venom of a species of Cnidaria, there are numerous clinical trials occurring which highlight the huge therapeutic potential for Cnidarian toxins (Table 2).

Venom components from Cnidaria contain an essentially unexploited source of novel bioactive compounds. Because of this many research groups have been working to understand these components to investigate if they have utility as a possible source of novel biotherapeutics [8,12,14,27,59-60,66,86-89].

Table 2: Potential Drug Leads Derived from Cnidarian Toxins. Taken from [15].

Toxin	Potential Drug Leads	Reference
TRPV1 Inhibitors	Analgesia, Anti-Tumour, Anti-Epileptic, Neuroprotective	[8,59,90]
Anti-BuChE	Neurodegenerative Diseases	[8,53]
KTxs	Analgesia, Antimicrobial, Anti-Obesity, Anti-seizure, Hypotension Treatment, T Lymphocyte Proliferation, Immunosuppression, Multiple Sclerosis Treatment	[8,59,89,91-92]
NaTxs	Anti-Tumour, Antiarrhythmia, Anti-seizure, Neuroprotective, Insecticidal	[59,89]
Kunitz Peptides	Anti-inflammatory, Neuroprotective	[59,93]
ASIC Inhibitors	Analgesia, Anti-Inflammatory	[59,89,94]
Actinoporins	Anti-Tumour, Anti-Parasitic, Leukaemia	[8,68,88,95]
EGF Targeting Toxins	Anti-Tumour	[66,96]

Treatment of Autoimmune disease

Sea anemone venom not only has an effect on sodium channels, it has also been shown to have an effect on potassium channels. In particular the voltage-gated potassium channel KV1.3 has been shown to be an important target for a number of autoimmune diseases as this is one of the potassium channels that is located on human T lymphocytes [97]. Kv1.3 has been demonstrated to be blocked very effectively by the peptide toxin ShK from the Sun Anemone (*Stichodactyla helianthus*). It is not completely specific to this channel as it also blocks some of the other potassium channels (Kv1.1, Kv1.4 and Kv1.6) but only weakly [98]. ShK has been made synthetically and a modification has been introduced to make it more selective for Kv1.3 and Kv1.1 as well by undertaking a K18A mutation which is just a one amino acid change. This then improves this peptide which makes it a better lead for targeting autoimmune disease [99]. Dalazatide a 37-amino acid synthetic peptide which was previously called ShK-186 and SL5 specificity targets Kv1.3 and is a treatment for psoriasis and other autoimmune diseases. The phase 1b trials were successful and now further larger studies are required [80]. Dalazatide was originally derived from the Sea Anemone *Stichodactyla helianthus* peptide Shk [100] which blocks the activation of the effector memory T-cells. Both BgK and ShK peptides discovered in the venom of the *Bunodosoma granulifera* potently block Kv1.3. Another peptide that acts on Kv1 and blocks the channel is OspTx2a which was discovered from *Oulactis* sp. [101]. Another peptide has recently been discovered from the same species is OspTx2b but this doesn't have any activity against Kv1 channels [96]. Kv1.3 is also a promising target for multiple sclerosis and ShK toxins and their analogs are being investigated further in this area [91,103].

Obesity and insulin resistance
Another disease that Kv1.3 inhibitors are also considered as a therapeutic target is obesity. ShK-186 could also be used in the treatment of obesity and insulin resistance as a study with mice showed a reduction in weight gain and enhanced insulin sensitivity in the treated animals [104].

Investigating blood disorders
The venom toxin PsTX-T of the Sea Anemone *Phyllodiscus semoni* can be utilised in the analysis of pathology, and in the development of therapeutic approaches in the condition haemolytic uremic syndrome (HUS) [89].

Potential for Cancer treatments
A Cnidarian venom that has been shown to have anticancer properties in liver and breast cancer is from the Giant Jellyfish *Nemopilema nomurai*. The human breast adenocarcinoma cell line MDA-MB-231 and the human hepatocellular carcinoma cell line HepG2 were utilised for these experiments. The researchers showed that this venom targeted Akt and mTOR signalling pathways which are dysregulated in cancer [105-106]. CqTX from the Box Jellyfish *Chiropsalmus quadrigatus* causes apoptosis in glioma cells [107]. The venom of the Atlantic Sea Nettle Jellyfish *Chrysaora quinquecirrha* contains a peptide called the Sea Nettle nematocyst venom (SNV) peptide which displayed substantial anti-tumour activity on an Ehrlich ascites carcinoma (EAC) tumour model [108].

It is not just Jellyfish toxins that show potential in the fight against cancer. Fractions of venom from the Snakelocks Sea Anemone *Anemonia viridis* have also been shown to have a cytotoxic effect on cancer cells [109]. A pore forming protein called Equinatoxin II from the Beadlet Anemone *Actinia equina* has shown to be toxic to leukaemic and Ehrlich ascites tumour cell lines [110].

A non-protein component of venom called Palytoxin from Soft Corals of the order Zoantharia and genus *Palythoa* and *Zoanthus* and the Sebae Sea Anemone *Heteractis crispa* has been shown to have anticancer activity against a number of cancer cell lines from head and neck cancer, Ehrlich ascites tumour and leukemic cells [111-112]. It is thought to act by distortion of actin filaments and cell death [113]. In contrast to the above Palytoxin has also been reported as a tumour promoter as it stimulates MAP kinase activity [114].

The Sun Sea Anemone *Stichodactyla. helianthus* produces another pore forming toxin called Actinoporin and this has been linked with an antibody in the treatment of colorectal cancer. Unfortunately, these chimeras produce non-specific toxicity, and more work needs to be done on this to develop it further [68].

Actinoporin RTX-A from the Sebae Sea Anemone *Heteractis crispa* has a cytotoxic effect on a range of cancer cells by inducing p53 independent apoptosis [95].

A compound called Sinularin from Soft Leather Corals *Sinularia flexibilis and Sinularia manaarensis* has been shown to have anticancer activity specifically against breast cancer cell lines compared to normal ones [115]. It works by affecting the cell cycle, causing DNA damage and programmed cell death and activation of PARP (poly(ADP-ribose) polymerase). It has also been

shown to be effective in human hepatocellular carcinoma cells utilising the similar mechanisms [116].

An Epidermal Growth Factor-like (EGF-like) peptide called Gigantoxin I has been discovered in the venom of the Giant Carpet Sea Anemone *Stichodactyla gigantea*. Gigantoxin I not only has homology with EGF it has been shown to have an effect on the cancer cell line A431 [96]. This cell line has an exceptionally high number of Epidermal Growth Factor Receptors (EGFR) which is the receptor for EGF and is an anticancer target [117]. Gigantoxin I also has an effect on Transient Receptor Potential Vanilloid Receptor 1 (TRPV1) channels [66].

Pore forming proteins EqTX-II and Bc2 from the Sea Anemones *Actinia equina* and *Bunodosoma caissarum* respectively had a cytotoxic effect on glioblastoma cells [118]. A toxin Sticholysin I (StI) from the Sea Anemone *Stichodactyla helianthus* has been used to produce immunotoxins to kill colon cancer cells [119-120].

Effect on sodium channels

It was back in 1969 when it was shown that a toxic component(s) from a Caribbean Sea Anemone *Condylactis gigantea* (Figure 2) caused a delay in the inactivation of sodium currents in the giant axon of a crayfish [121]. Voltage gated sodium channels are targeting by a number of Sea Anemone venoms affecting receptor site-3 of the alpha subunit of this channel which causes inactivation thus affecting the gating of the sodium channel [122]. Toxins Av1, Av2 and Av3 originally isolated from *Anemonia viridis* have now been found in a number of Sea Anemones and shown to inactivate sodium channels [122]. Gigantoxins II and III from the Sea Anemone *Stichodactyla gigantea* have activity on sodium channels [96].

Cardiovascular disease treatments

Anthopleurin A and B are peptides from the Giant Green Sea Anemone *Anthopleura xanthogrammica* and have an effect on sodium channels and have shown initial promise as drugs for use in cardiovascular disease by strengthening the force of the heartbeat (positive inotrope) [123-124]. Anthopleurin C was isolated from the Aggregating Sea Anemone *Anthopleura elegantissima* and has shown similar action to Anthopleurin A. Hk2a is another peptide toxin which also has a very similar structure to Anthopleurin C and the neurotoxin 1 [125]. A sodium ion channel modifier AdE-1 discovered in the Sea Anemone *Aiptasia diaphana* is a novel cardiotonic peptide [126]. It acts by significantly impeding current inactivation with no significant effect on current activation. The Sea Anemone *Anemonia viridis* peptide ATX-II inhibits the inactivation of the sodium channel in the heart and could be promising as an antiarrhythmic drug [127].

New approaches to treat liver disease
There is another isoform of Anthopleurin called Anthopleurin Q discovered in the venom of *Anthopleura xanthogrammica* (Figure 3) which has been shown to have an effect on rat liver cells and is being investigated due to its protective effect on liver injury [128]. It is thought to do this from selectivity delaying outward potassium currents in hepatocytes (liver cells).

Alleviation of pain by the development of new analgesics
Obtaining acceptable pain control is still a significant health problem and new drugs are needed to treat pain which are more effective with less side effects. Venom components could deliver new treatments as they do affect a number of pain targets [129]. TRPV1 and Acid-Sensing Ion Channels (ASICs) are important drugs in drug discovery for the treatment of pain and several components of Sea Anemones have been found to block these receptors. From the Sea Anemone *Heteractis crispa* was discovered TRPV1 inhibitors named APHC1, APHC2 and APHC3 which could be utilised as analgesics [8,89,130-132]. An N-acylamino acid called Bunodosine was discovered in the venom of the Sea Anemone *Bunodosoma cangicum* could be utilised as an analgesia as it is thought to activate serotonin receptors [133]. The peptide PhcrTx1 from the Rock Flower Sea Anemone *Phymanthus crucifer* has been found to be an ASIC inhibitor and could be utilised as an analgesia [134]. It also acts on voltage gated potassium channels (Kv) but with a much-reduced effect compared to the ASIC channel. From the Painted Sea Anemone *Urticina grebelnyi* the peptide π-AnmTX Ugr 9a-1 was discovered and was shown to specifically inhibit the human ASIC3 channel in a *Xenopus* oocyte model [135]. The Sea Anemone *Anthropleura elegantissima* toxin $APET_x2$ inhibits inflammatory pain through the ASIC3 receptor [59,94,136].

Peptides from the Sea Anemone *Heteractis crispa* have Kunitz peptides HCRG1 and HCRG2 that possess Kv1.3 blocking activity and have anti-inflammatory properties. In particular HCRG1 is very effective in reducing the synthesis of tumour necrosis factor (TNF) alpha [93,137].

Novel therapeutic strategies for Epilepsy
As mentioned above, APETx2 blocks ASIC3 and this toxin has also been used to better understand the mechanisms involved in epilepsy [138].

Antimicrobial activity
The diterpenes Flexibilide and Sinulariolide from Soft Coral *Sinularia flexibilis* have shown antimicrobial activity [139]. A peptide called Hydramacin-1 was extracted from the freshwater Hydrozoan *Hydra magnipapillata* [140] and was found to have antimicrobial activity which includes activity against antibiotic resistant bacteria and fungi as well as other gram positive and gram-negative bacteria. Periculin-1 is another antimicrobial peptide from the Hydrozoan *Hydra vulgaris* [141]. There are many other antimicrobials that have been discovered. A comprehensive review of this area has been written elsewhere [49].

Insecticides
Insecticidal toxicity has been enhanced by fusing Cry1Ac of *Bacillus thuringiensis* with a neurotoxin that is toxic to Cockroaches and Crustaceans but doesn't affect Mammals called Av3 from the Sea Anemone *Anemonia viridis* [92].

Novel treatments of neurodegenerative diseases

Crude venom from the tentacle material of the Mediterranean Jellyfish *Pelagia noctiluca* has been shown to inhibit butyrylcholinestrasic activity so has the potential to be utilised in the treatment dementia and dementia related diseases [142]. Although the component or components in the venom haven't been identified yet [143].

SUITABILITY AS DRUG LIKE MOLECULES

Development of drug like molecules from Cnidaria venoms

Studies of toxins that have been suitably purified for drug discovery have, with few notable exceptions, only investigated target engagement and often selectivity. They negated to investigate other drug like properties such as absorption, dispersion, metabolism, and excretion (ADME). Key criteria for measuring absorption and dispersion of circulating drugs are C_{max} and T_{max}. C_{max} is the maximum plasma concentration and T_{max} is the time taken to reach that concentration. In the envenomed host natural selection has led to venoms with high C_{max} and short Tmax from subcutaneous injection as these will subdue the prey the fastest or repel predators faster. A slow acting venom with a long T_{max} would give the prey time to escape from the soft bodied Cnidarian predator. On the other side the predators would have already consumed the Cnidarian prey before the venom takes effect, thus being too late for both animals. These pharmacokinetic (movement of drugs in the body) properties are not only useful in the wild but also fit well for drug development. Metabolism and excretion of the venom is presumed to be after the key events in envenomation and thus presumably are not affected by natural selection. The other aspects of the venoms such as their 3D structure and modification to engage targets do indirectly affect their metabolism and excretion parameters in a way that could be useful for drug discovery. ADME properties are key criteria for any drug to be considered worth the investment to move from preclinical research to clinical drug trials. This is because without suitable ADME properties the molecules will either fail at getting to the target, or not be at a sufficient concentration for long enough to elicit the desired pharmacological effect. Modern humans have evolved to live on a diet rich in protein, which means that the oral route of administration for peptide drugs is a challenging one. However, progress is being made in this field, such as the development of Exanta™ (Ximelagatran) produced by AstraZeneca from a cobra venom peptide. Although it was not successful in clinical trials it has shown oral venom peptide drug delivery is possible [144]. The cone snails are probably the best studied marine invertebrate venoms as several of their disulphide bridges peptides have drug like properties and one [145] has made it to become a licenced therapeutic. Synthetic modification of the hydrogen bond structure of alpha-conotoxin Vc1.1 permits oral bioavailability [146]. More advances in peptide engineering are likely to lead to a greater number of orally available peptide therapeutics.

Preclinical evaluation of Shk toxins for clinical use in autoimmune disease has used once daily sub cutaneous dosing of the peptide solution due to the plasm half-life of ~ 50 minutes [91]. However it appears that significant amounts of the peptide bind to plasma proteins and act as a reservoir prolonging target engagement for many hours [91]. Further stability from the native peptide was achieved though addition of C-terminal amide, non-hydrolysable C-terminal modifications [91]. Because small peptides derived from venoms are readily synthesised chemical

they can be easily modified such as using D enantiomers to protect from proteases [147]. C-terminal amides to appear as natural stability modifiers in venom peptides, such those from cone snails [148] but have yet to be reported naturally occurring for Cnidaria. Post translational substitution of the carboxylic acid group of peptides for amides naturally protects against destruction by carboxypeptidase [91] In clinical use, Shk-186 (dalazatide) achieved rapid plasma exposure (T_{max} ~30 minutes) from subcutaneous injection to Psoriasis patients but was effective with doses 29 days apart [100]. However, Shk-186 has a short plasma half-life where >90% of the circulating dose is lost in two hours, there is slow release of the remainder from plasma protein binding [149]. Shk-186 also reduces immune-infiltration of transplanted kidneys in animal models, however 100ug/kg was needed to be injected twice daily to achieve therapeutic activity [91].
As for excretion peptide drugs, including shk suffer from rapid renal clearance [100], however simple addition of polyethylene glycol chains, called PEGylation, improves the pharmacodynamics by reducing renal clearance, such as has been demonstrated with a snake venom serine protease [150].

CONCLUSION

Despite being the oldest and most speciose venomous clade, research into the utility of Cnidarian venoms lags behind that of other taxa. This is predominantly due to challenges in sourcing and extracting such venoms. Recent research and improvements in aquaculture are opening up access and understanding of these amazingly useful proteins. There is no doubt that in the coming years a greater understanding of Cnidarian venom composition and drug like properties will add to the utility of this fascinating clade. The first Cnidarian venom clinical candidate dalazatide, is showing promise in autoimmune disorders due to long target engagement and useful pharmacokinetics. The future of venom research will see more focus on drug like properties such as absorption, dispersion, metabolism and excretion in addition to potency and selectivity. This will fuel a great increase in our ability to fight challenging disease with novel therapeutics. No doubt there are many more drug like molecules and research tools within the venoms of these incredible animals. Surely, even for selfish reasons, we should be doing more to protect our aquatic habitats and the remarkable species within.

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Figures

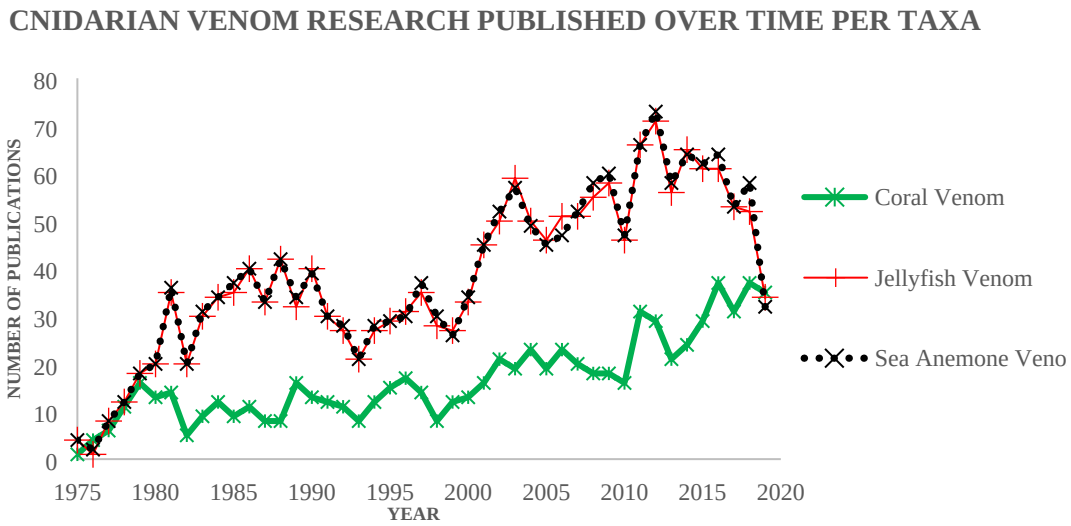

Figure 1: The annual publication rate for each of the major Cnidarian lineages between 1975 and 2019. Data obtained through a PubMed search of each taxon 'AND venom'. Taken from [15].

Figure 2: The Caribbean anemone *Condylactis gigantea* in captivity at Venomtech Ltd. Venom from this species contains toxins useful in modulation of Sodium channels. Photo by Steven A Trim.

Figure 3: *Anthopleura xanthogrammica* Anthopleurin from this species has been shown to protect the liver from injury. Photo by Phillip Robinson