Novel Pharmacological Targets From Indian Cone Snails

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Abstract: The oceans are a source of combinatorial library of unique natural products, 'not found in the terrestrial environment'. Marine invertebrates such as sponges, molluscs, bryozoans, tunicates (Urochordata) and their associated microorganisms are the major representatives of promising bioactive compounds. Among these, the predatory molluscan cone snails have evolved with highly structured small and complex array of peptides (more than 50,000) linked to their prey capture and defence. These peptides have become a valuable source of neuro pharmacological targets as many of them selectively modulate ion channels and transporters. A group of scientists from United States, Europe, Australia, Israel and China has characterized drugs for neuropathic pain and pharmacological targets from the peptides of a few cone snail species. Several are now in clinical and preclinical development. Less than 1% of the cono peptides are pharmacologically characterized. India has a diversity of 20-30% of total cone snail species distributed worldwide. A group of Indian Scientists has made promising drug discovery programs from *Conus* peptides. This review will focus on the *Conus* peptides from Indian cone snails species, their neuro pharmacological targets and future directions.

Keywords: Indian cone snails, C*onus monile*, fingerprint of conotoxins, pharmacological targets, voltage gated sodium channels, calcium channels, potassium channels.

INTRODUCTION

The cone snails of the genus *Conus* (conidae) are a large group of recently evolved marine neogartopod molluscs. They use highly developed envenomation apparatus to inject neurotoxin which paralyses the prey within seconds. The major constituent of the venom is a complex mixture of peptides called conopeptides small disulphide –bonded evolved to rapidly immobilize the prey. The evolution of conotoxin in the venom of predator snails may be influenced by selective pressures imposed by the nature of the prey, with peptide mixtures from piscivorous , molluscivorous, and vermivorous snails exhibiting differences [1]. Each venom type contains a unique array of over 100 different peptides. Many of these peptides are post-translationally modified which include bromination of tryptophan, C terminal amidation, isomerisation of L to D aminoacid, hydroxylation of proline, sulfation of tyrosine residues, glycosylation of serine and threonine residues and γ carboxylation of glutamyl residues [1]. The classification of conotoxins has relied on the distribution of Cys residues in the primary structure, the nature of the disulphide pairing topology and the functional attributes of the peptides [2, 3]. The specific targets, defensive mechanism, tranquilizing effects of conotoxins are poorly defined. This broadly evolved pharmacological target provides a unique source of therapeutic agents such as ω - MVIIA (Prialt® / Ziconotide) [4] and have become new research tool for neuro pharmacological scientists.

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Conotoxins are genetically encoded propeptides, which following expression and cleaved by specialized endoprotease, produce the mature venom peptide [5]. Their small size (5-30 amino acid residues), relative ease of synthesis, structural stability and target specificity make them ideal pharmacological probes [6]. It is estimated that more than 50,000 conopeptides have evolved, with only approximately 0.1% characterized pharmacologically. [5]

The cone snails of India are of particular interest because of the biodiversity of about 77 species, 21 of which are unique [7]. A multi-institutional collaborative research program was initiated by the Indian Institute of Science and National Center for Biological Sciences to investigate the chemistry and pharmacological targets of the peptides from cone snails off Indian coast. This review focuses on some of the novel pharmacologically important peptides from Indian cone snails.

SPECIES ABUNDANCE

A survey on the diversity of cone snails revealed its abundance in Rameshwaram, Mandapam, Tuticorin (Gulf of Mannar), Portonovo, Kollam from east coast and Mumbai waters of west coast of India. More than 50 species have been collected during the survey. Of these only 10 species were abundant and they were found to be abundant [8]. The most common Indian cone snails are *Conus araneosus, Conus monile, Conus betulinus, Conus virgo, Conus loroisii, Conus amadis, Conus achatinus, Conus inscriptus, Conus textile* and *Conus hyaena*. The systematic study of the radular teeth morphology of Indian cone snails based on their feeding habits [8] enabled the study of diversity of peptide composition in Conus species with diverse pharmacological targets.

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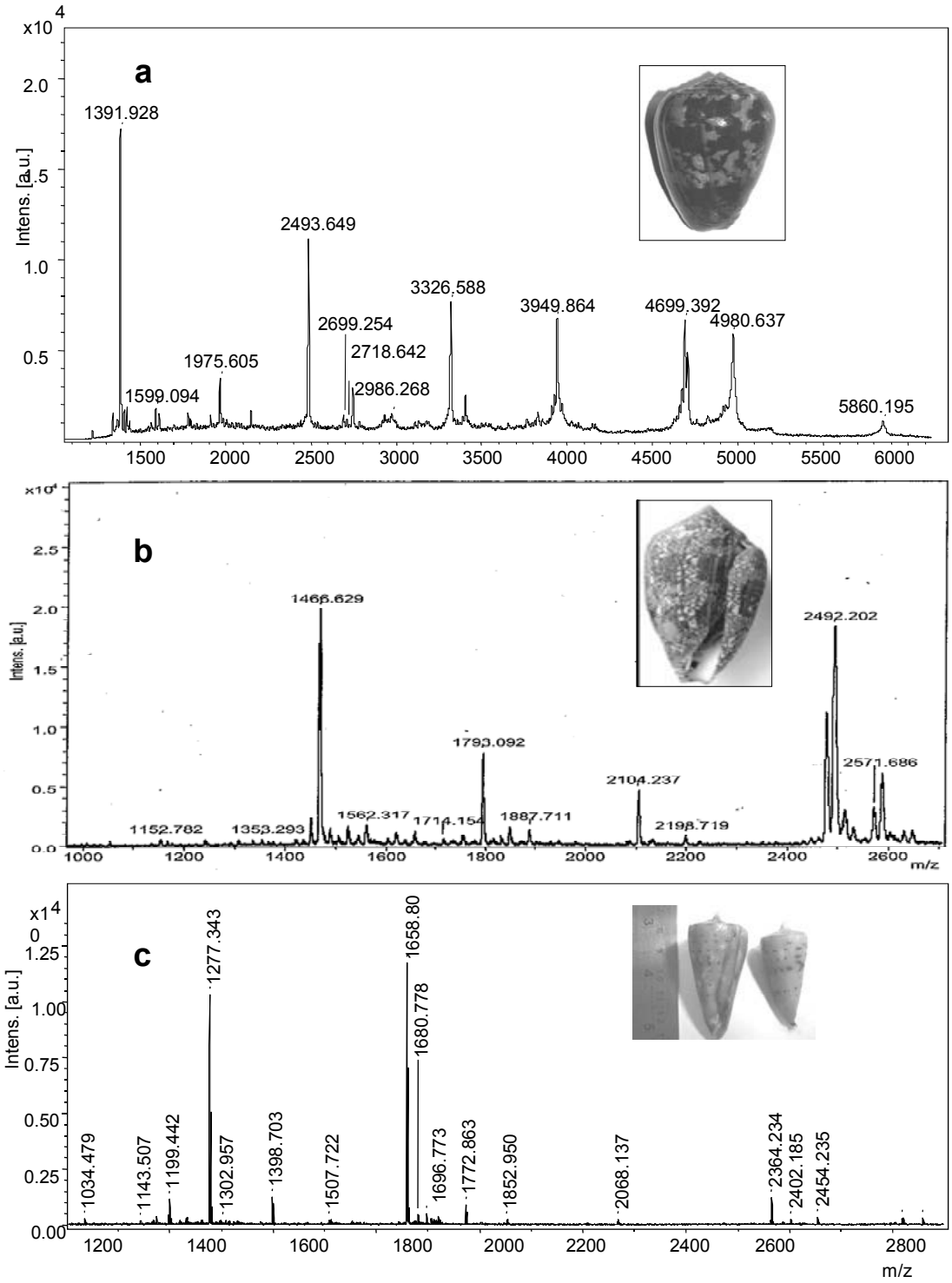

Fig. (1). Fingerprint of cone snail venom **a**) *Conus striatus* (piscivorous) **b**) *Conus textile* (Molluscivorous) **c**) *Conus monile* (vermivorous).

FINGERPRINT OF CONOTOXINS

The fingerprint is nothing but a mass spectrometric profile of crude venom extracted with organic solvents and the spectrum has been acquired with different types of matrix by using MALDI mass spectrometry. The fingerprinting of conotoxins have given the overall peptide composition of the respective toxin and lead to further isolation and purification of the particular peptide of interest. The fingerprint of global reduction alkylation of crude venom by using MALDI MS helped to identify the δ and ω conotoxin by comparing cDNA sequencing of mature toxins in order to use de novo sequence determination. In addition, the Vi 1359 and Vi1361

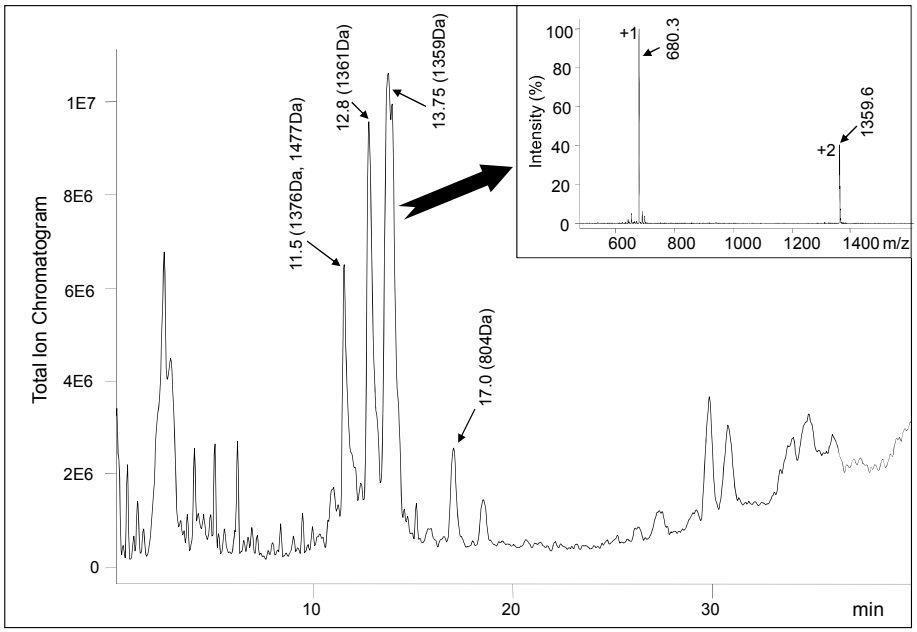

Fig. (2). Total ion chromatogram obtained from LC- ESI /MS of methanol extract of crude venom from *C. virgo*. ESI mass spectrum of two major peaks in HPLC profile has shown inset *Vi* 1359 & *Vi* 1361 [18].

(T super family peptides) peptides were isolated and sequenced by Tandem Mass spectrometry techniques (Denovo sequencing) (Fig. **2**) from vermivorous cone snail *Conus virgo*. The pharmacological target studies are still being carried out. But, in animal model studies (with mice), it induced antinociceptive activity (Table **1**). The fingerprint of *Conus straitus* (piscivorus), *Conus textile* (molluscivorous) and *Conus monile* (vermivorous) are shown in (Fig. **1a**, **b**, **c**).

CALCIUM CHANNELS

Toxins that selectively target voltage-sensitive calcium channel have mainly been isolated from cone snails whereas, a number of nonselective inhibitors has been found in both snake and spider venoms [4]. ω conotoxin which is acting on N-type calcium channels play a key role in pain transmission by controlling neurotransmitter release at spinal synapse and thus can act as a gate keeper for responses to sensory pathway activation [9]. ω conotoxin have been identified from different species of fish hunting snails, including GVIA from *Conus geographus*, MVIIA from *Conus magus* and CIVD from *Conus catus*. Though there is a sequence homology among ω conotoxins, MVIIA and MVIIC target different voltage sensitive calcium channels, whereas ω conotoxin GVIA and MVIIA inhibit the same channel subtype despite remarkably low sequence homology [10]. Ac 6.4, a ω conotoxin was isolated from fish eater *Conus achatinus* from Gulf of Mannar , South East coast of India and sequenced by mass spectrometry and cDNA sequencing (Fig. **3**) [11]. Interestingly, the contryphans Lo 959 and Am 975, which are isolated from Indian cone snails *Conus loroisii* (worm eater) and *Conus amadis* (mollusc eater), are having single disulphide bond exhibit very high homology (exception of residue 3, pro in Lo 959 and hyp in Am 975) [12] (Table **1**). These contryphans are identical to contryphan-P, previously isolated from *Conus purpurascens* (a piscivorous). Lo959 and Am975 undergo slow conformational interconversion under reverse phase chromatographic conditions, a characteristic feature of all contryphans and **e**lectrophysiological studies of these two contryphans revealed that the former enhances the Ca^{2+} and latter appears to diminish the magnitude of the whole cell Ca^{2+} current (Table **1**). It was observed [12] the conservation of mature contryphan sequences across the *Conus* species is much higher than the multiply disulphide bonded conotoxins [12]. Hence, the three dimensional structural variability of contryphans which are having multiple conformational states provide a mechanism for targeting diverse receptors. The intravenous injection of contryphan 959 leads to antinociceptive, hypoactive state in mice and reduced gill movement in fish (Table **1**).

SODIUM CHANNEL MODULATORS

Voltage gated sodium channels play a key role in controlling neuronal excitability. Based on their susceptibility to block by the puffer fish toxin (TTX), sodium channels can be divided into TTX sensitive (TTX-S) and TTX resistant (TTX–R classes). Nine homologous α subunit subtypes have been identified ($Nav_1$1.1-1.9) [13]. Several clinical states such as pain, stroke and epilepsy are implicated by these subtypes of Sodium channels [4,14]. Given their critical role in the central and peripheral nervous system, it is not surprising that cone snails have evolved a number of different ways to target this ion channel class. Among 14 types of conotoxin, δ conotoxins have been shown to inhibit voltage gated Na^+ channel inactivation [10]. Am 2766 from *Conus amadis* (Molluscivorous) found off South east coast of India shows an effect on brain r Nav1.2 voltage gated sodium channels expressed in CHO cells [15]. These observations have coincided with oδ conotoxin of *Conus glomeriamaris* (Gm VIA), *Conus nigropunctatus* (NgVIA) and *Conus*

Table 1. Novel Pharmacological Target Peptides Being Developed From Indian Cone Snails

S.No	Name of the species	Name of the peptide	Sequence	Target/Activity	Type of Cono-toxin	Ref
1	*Conus virgo*	Vi1359	Z*CCITIPECCRI-NH_2	Antinociceptive, tilted swimming in fish (unpublished)	T –Super family	[19]
		Vi1361	Z*CCPTMPECCRI-NH_2	Under investigation	T - Super family	Unpublished
		Vi 805	H_2N-WPGPWY-OH	Under investigation	-	Unpublished
2	*Conus monile*	Mo1659	FHGGSWYRFPWGY-NH_2	Non inactivating K^+ channel	-	[17]
		Mo 1274	GNWCCSARVCC	Under investigation	T Super family	Unpublished
3	*Conus amadis*	Am2766	CKQAGESCDIFSQNC-CVGTCAFICIE-NH_2	Voltage gated Na+ channel (rNa1.2a)	δ conotoxin	[20,21]
		Am975	GCOWDPWC*-NH_2	Voltage gated Ca^{2+} Channel	Contryphans	[12]
4	*Conus loroisii*	Lo959	GC*PWDPWC*-NH_2	Voltage gated Ca^{2+} Channels antinociceptive	Contryphans	
5	*Conus araneo-sus*	Ar1447	-	Excitory behaviour, scratching and digging	-	Unpublished
		Ar1859	-	Sleeper peptide, antinociceptive	-	Unpublished
		Ar1813	-	Antinociceptive	-	Unpublished
6	*Conus achatinus*	Ac6.1	DECFSPGTFCGIKPGLCCSAW-CYSFFCLTLTF	Voltage gated Na^+ channel	δ conotoxin	[11]
		Ac6.2	DECYPPGTFCGIKPGLCCSETCFPFV CLSLEF	Voltage gated Na^+ channel	δ conotoxin	
		Ac6.3	YECYSTGTFCGGVNGGLCCSNLCL FFVCLFS	Voltage gated Na^+ channel	δ conotoxin	
		Ac6.4	CKGKGASCSRTMYNCCTGSCNRGKCG	Voltage gated Ca^{2+} Channels	ω conotoxin	

textile (Tx VIA) showed homology in cysteine pattern. The 3D structure of δ Am2766 [15] has a similar structure of O – type conotoxins and characterized by the presence of an 'inhibitory cysteine knot' (ICK). Compared to δ TxVIA and δGmVIA, δ Am2766 exhibits higher hydrophobicity. δ Am2766 was found to be active in Nav1.6 mediated Na+ currents. The solution structure of δ Am2766 [15] provides insight into the structural determinants important for their activity VGSC, although the specific residues involved remain to be identified. δAm2766 is a new addition to the most extensively studied scorpion α toxin and sea anemone toxins, which inhibit Na+ current inactivation by binding to the receptor site 3 [15] . Competitive binding experiments with this δ Am2766 may be useful in elucidating the exact mechanism by which the δ conotoxins inhibit fast inactivation of Na^+ currents. In addition, two δ conotoxins were isolated from the fish hunter *Conus achatinus* and sequenced by using both tandem mass spectrometric technique and cDNA sequencing [11].

POTASSIUM CHANNELS

Compared to the diversity K^+ channel inhibitors produced by scorpion and sea anemones, the cone snails appear to have evolved relatively few peptides active at this physiologically important target [16]. κ conotoxin Mo1659 was isolated from the vermivorous *Conus monile* [17] and sequenced by edman sequencing. This novel unusual sequence with a preponderance of aromatic residues and absence of disulphide bridges resembles no cysteine residues. This is the first non disulphide bonded peptide which influences the K^+ currents

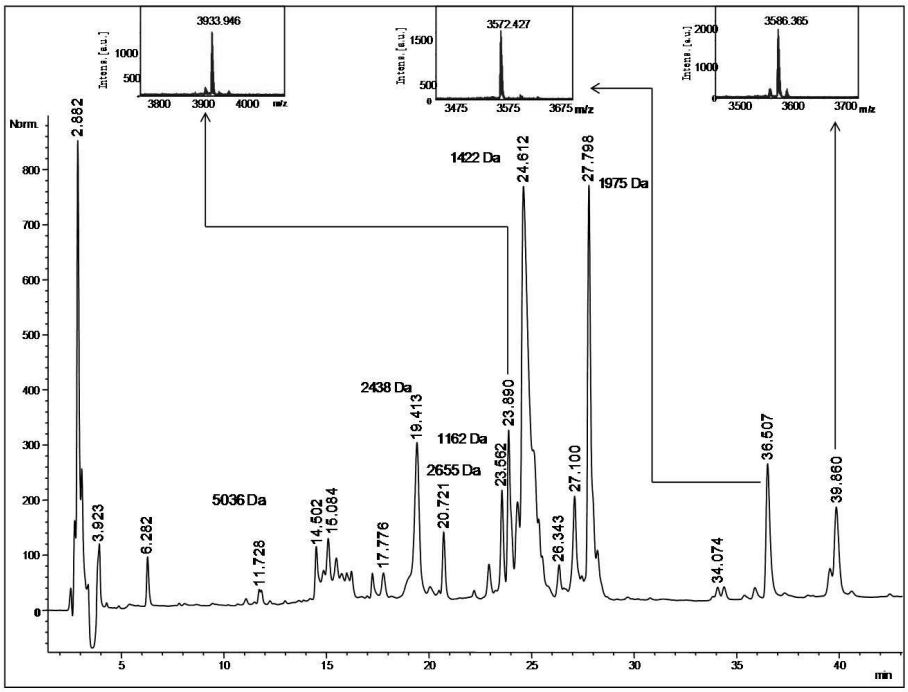

Fig. (3). HPLC profile of *Conus achatinus* crude venom [9].

in neurons whereas PVIIA (κ-PVIIA), a peptide containing 27 aminoacids, cysteine rich (disulphide bridges) toxin is the first κ conotoxin that inhibits shaker potassium channel [18].

CONCLUSION AND FUTURE PROSPECTS

Because of the high selectivity of conus venom peptides , they have been proved to be useful tool for *in vitro* and *in vivo* proof of concept studies. The vast array of combinatorial library of peptides remain pharmacologically uncharacterized and enormous opportunity remains to identify new research and potential therapeutics from amongst the highly diverse venoms of Indian Conidae (77 sps) [7]. The exploration of cone snails for the novel drug development is very scanty. Hence, the high throughput screening strategies are expected to accelerate the drug discovery process of new conotoxin. However, a number of issues such as safety, delivery and pharmacokinetics are need to be addressed.

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