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# A brief review on peptide toxins from Cone snail (CONOTOXINS)

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## ABSTRACT

Cone snails are marine gastropods serving as a source for the production of various bio-active compounds. These bioactive compounds are used by pharmaceutical industries for developing drug formulations with less toxic effects. Cone snail peptides are of significant importance, as these peptides are exhibiting large chemical diversity and potency towards targeting specific membrane receptors. Conopeptides are venom peptides used by cone snails for prey capturing and defense. These peptides are capable of specifically modifying various ion channel transporters and membrane receptors. Conopeptides contains about 60-80 amino acids and is mostly studied at mRNA and protein level. Conopeptide synthesis proceeds in such a way, that the signal and pro-peptides are cleaved by proteolysis yielding the final matured peptide. These final matured peptides have small structural units, capable of modifying specific ion channels of the mammalian nervous system. Conopeptides have high potential towards ion channels, making them as an ideal drug lead.

Key Words: Pharmaceutical, Cone snails, Conopeptides, Proteolysis, Protein.

## 1. INTRODUCTION

Conopeptides are the most vital compounds, hundreds of these different conopeptides have been observed in the venom of one cone snail species however, only few of these conopeptides have been found in more than one species [1]. Marine predatory cone snails are capable of producing series of venom peptides, referred to as conopeptides. These conopeptides are used by the cone snails for capturing prey. However, more than 700 conus species, producing conopeptides, forms a large library of bioactive peptides [2].

Conopeptides are synthesized as long peptide precursors containing a N-terminal signal peptide (~ 20 AA) and a pro-peptide (20–30 AA) sequence. These signal peptide sequences target the conopeptide secretion, while the pro-peptide assist in proper folding. Both the signal and the pro-peptide are further cleaved by proteolysis, yielding the final matured peptide [3]. Signal sequences are conserved, but the mature toxin sequences, at the C-terminal are highly divergent. Final matured peptides are capable of having high frequency towards post-translational modifications. Due to its high potency towards ion channels, these peptides gain more attention in the field of neuropharmacology as drug leads.

Transcriptomic studies of *Conus miles, Conus tribblei, Conus victoriae, Conus marmoreus* were found with different conopeptide transcripts from one cone snail species. Prialt, a synthetic conopeptide was approved for treating chronic pain.

Conopeptides are mostly studied at the mRNA and peptide level, but only a little is known about their gene structure. Mature peptides of the O1-superfamily are each coded in a different exon separated by long introns.

Each region of the pre-pro-peptide sequences were diverged at very different rates. Signal sequences are highly conserved, even at the nucleotide level within the superfamily (almost no synonymous substitutions), but the mature peptide region had mutation rate that is more than ten times higher [4].

For most conopeptide superfamilies (I1, M, O2, O3, P, S, T), gene structure is similar to that of O1 superfamily. First exon has coding sequences for 5' untranslated region (UTR), followed by signal peptides, and also few codons of pro-peptide. Second exon codes for pro-peptide and the third exon for mature peptide and 3' UTR. These three exons are separated by introns that are more than three kilo bases (kb) long. I 2- superfamily conopeptide genes also have three exons and two introns, however, the order of their functional part is quite different. In this case, the pro-peptide comes after the mature peptide in the last exon and is termed a post-peptide [5].

Intron sequences are conserved within superfamilies among different species, just like the signal peptide sequences and this is known as conopeptide diversity. Thousands of peptides with different masses were detected in the cone snail venom using modern ultra-sensitive mass spectrometry technologies [6].

Each cone snail species expresses roughly one hundred to several hundred conopeptide genes. These conopeptide genes are thought to be under diversifying selection. Alternative splicing have been detected for at least one conopeptide. Main mechanism for increasing the variability of conopeptides appears to be the post-translational modification. Maturation process of conopeptides includes cleavage of both the signal and pro-peptide along with various other processers such as the formation of disulfide bridges, C-terminal amidation, and hydroxylation of proline at its C-4 site [7].

To date, 16 different naturally occurring post-translational modifications have been described for conopeptides. Cleavage of the N-terminal pro-peptide is not site-specific and amino acids can also be cleaved from the C-terminus. Both alternative post-translational modifications and cleavage of the pre-pro-peptide are reported to be observed. For instance, venom peptide from *Conus marmoreus* contains an average of 20 different and a maximum of 72 unique masses per precursor sequence [8].

## CONOPEPTIDE CLASSIFICATIONS

Based on their sequence similarities, cysteine residues present in conopeptides are classified into gene superfamily, cysteine framework and pharmacological family [9].

## 2. GENE SUPERFAMILIES

Conopeptides are classified into gene superfamilies based on their signal sequence similarities [10]. Evolutionary evidences shows that members of different gene superfamilies are genetically and evolutionarily divergent [11]. There are 26 superfamilies in the conopeptides reference database, Cono Server (www.conoserver.org), however, 35 are listed in a recent review about conotoxin superfamilies (Robinson and Norton 2014). Main difference comes from the fact, that conoserver does not classify cysteine-poor (two cysteines or less) peptides into superfamilies. They are classified separately into classes, however, differentiation between cysteine-rich and cysteine-poor peptides have shown to have no phylogenetic meaning [12].

Some cysteine poor conopeptides previously classified into families, have been re-classified into superfamilies, along with the cysteine-rich conopeptides (conomarphins and contryphans have moved into the M and O2 super-families, respectively) [13]. Other cysteine-poor conopeptides have unique signal sequences and are placed within their own superfamilies (conantokins and contulakins can now be referred to as superfamily B and C, respectively) [14]. Every transcriptomic study revealed that conopeptide sequences cannot be placed into any of the existing superfamilies. Some authors confidently declared new superfamilies, while others assigned them to temporary superfamilies. There are currently 13 superfamilies from *Conus californicus* in conoserver that are termed "divergent". There is no question if the conopeptides within these divergent super-families exist. Robinson and Norton also noted that superfamilies identified using only one or two cone snail species should be considered putative [15].

## 2.1 A SUPERFAMILY

A-superfamily is considered as the one well characterized family, owing to the arrangement of cysteine framework I (CC-C-C). This cysteine framework pattern is primarily similar to that of alpha-conotoxin family, acting as antagonist for nicotinic acetylcoline receptors (nAChRs). These nicotinic acetylcoline receptors are ligand gated ion channels, which are capable of performing a range of physiological and pathophysiological processes such as, muscle contraction, pain sensation and nicotine addiction**.** A-superfamily conotoxins were isolated from six precursor cone snail species, most of them were encoded by α-conotoxins and other peptides belonging to a family of excitative peptides called as kappa – conotoxins, targeting voltage gated ion channels.

Alpha conotoxins are nicotinic antagonists containing 13-19 amino acids and are considered to be the larger conopeptides. A4.2 conopeptide was isolated from *Conus aurisiacus* and has 30 residues with an average molecular weight of about 4232.92.

Two penta-decamer conopeptides, Ac1.1a and Ac1.1b strongly have α1-δ subunit interference instead of α1-γ binding site on mouse nicotinic acetycoline receptor [16]. A peptide Ai1.2, was isolated from *Conus ammiralis* (Fig. 1), belonging to A-superfamily of conotoxin consisting of 18 amino acid residues (PECCSDPRCNSTHPELCG). It has an average molecular weight of 1942.71. It was reported that this peptide inhibits the calcium channels in rat. CcTx activates the neuronal voltage gated sodium channels but its precise molecular target and mode of action is yet to be elucidated (Le Gall et al., 1999). However, serine residues present at the site 7 on this peptide was glycosylated [17].

## 2.2 B1 SUPERFAMILY

Conantokin-G, a gamma-carboxyglutamic acid isolated from *Conus geographus*, has 18 amino acid residues [GE(Gla)(Gla)LQ(Gla)NQ(Gla)LIR(Gla)KSN(nh2)] with an average mass of 2264.21. This species belongs to class II of cysteine family, and mainly feeds on *piscivorous* species. Conantokin-G was found to be active against the NMDA glutamate receptors [18], and more precisely acts as NR2B-selective competitive antagonist [19]. It is a polypeptide consisting of five gamma-carboxyglutamic acid residues acting as NMDA-type glutamate receptor antagonist [20]. Calcium binding strategies were identified by applying specific genetic algorithm. However, when calcium (Ca2+) binds to gamma-carboxyglutamic acid, conantokin G undergoes conformational change from a distorted curvilinear 310 helix to a linear α-helix [21]. Conantokin-L isolated from *Conus lynceus* has 20 amino acid residues [GE(Gla)(Gla)VAKMAA(Gla)LAR(Gla)DAVN(nh2)]. an average mass of 2207.26. It feeds on *piscivorous* species and was active against the NMDA glutamate receptors. Conantokins are N-methyl-D-aspartate receptor antagonists [22]. Conantokin-T2 isolated from *Conus tulipa*, has 12 amino acid residues (GEEEYQKIVGKI) with an average mass of 1391.73. *Conus tulipa* is a *piscivorous* cone snail and comprises of both paralytic and non-paralytic conotoxins [23].

## 2.3 B2 SUPERFAMILY

It was reported that the first member of this gene superfamily was found to be highly expressed one in Conus literatus venom gland cDNA, library and coined to be a high frequency protein-1. A clearly defined sequence of this gene superfamily was identified in the transcriptome of Conus consors and was even matched several linear peptides in the venom. Confirmation of this high frequency peptide, in conus came from the recent identification of the several similar sequences in the venom gland transcriptomics of three cone snails such as, C. geographus, C. Victoriae, and Conus bullatus. Its activity has not been reported yet, and it has been assigned under "B2 – superfamily" in the conoserver.

Conantokin-T31 isolated from *Conus tulipa* (Fig. 3), has 34 amino acid residues (GEELEERSHHSKFNGDSDNSPFQSEDGLETSWTS). It is a *piscivorous cone snail* having an average mass of 3839.87. It was found that this superfamily was highly expressed and termed as high frequency protein-1[24].

### 2.4 B3 SUPERFAMILY

**VX24a was isolated from the venom of the vermivorous cone snail, Conus vexillum. This venom peptide has 40 amino acid residues,** (VRCLEKSGAQPNKLFRPPCCQKGPSFARHSRCVYYTQSRE) **having an average mass of** 4625.31.

Conotoxins (Ctxs) targeting ion channels, are used as an ideal tool for probing nervous system function. αB-conotoxin VxXXIVA isolated from *Conus vexillum*, has unique arrangement of cystein residues [25]. Mechanism of αB-VxXXIVA conopeptides, reported that it is acts as an antagonist for nicotinic acetylcoline receptor (nAChR) having great potential towards α9α10 subtype. Its structure was identified using 1H nuclear magnetic resonance (NMR) spectra, indicating that these isomers are poorly structured.

### 2.5 C SUPERFAMILY

Contulakin-G was isolated from *Conus geographus*, and have 16 amino acid residues (ZSEEGGSNA(gTr)KKPYIL) with an average mass of 2070.19. *Conus geographus* is a *piscivorous* cone snail and was reported to have the Motor control associated dysfunction in mice. This resulted when, contulakin-G was administered intravenously into mice. This peptide binds to humans and rat neurotensin receptors, further this peptide's non-glycosylated form is about 10-fold less active [26].

### 2.6 D SUPERFAMILY

Alpha D conotoxin are noncompetitive antagonists of the neuronal nAChRs. This conotoxin was reported to have specific selectivity towards alpha7- and beta2 subtypes.
Characterization of three conopeptides alpha-Dconotoxins now named as, VxXXA, VxXXB, and VxXXC, occur as dimers, acting as opposers for alpha7 and alpha3beta2 nAChRs. Sequences of these peptides have been identified through a combination of Edman N-terminal sequencing and tandem mass spectrometry.

PiXXA isolated from *Conus princeps* (Fig. 5) comprises a amino acid sequence of about 50 residues(AVKKTCIRSTOGSNWGRCCLTKMCHTLCCARSDCTCVYRSGKGHGCSCTC)with an average mass of 5364.27. Conopeptide F7-3 was isolated from *Conus princeps*, and purified by Reverse phase high performance liquid chromatography (RP-HPLC). It has an amino acid sequence with an average mass of 10,735.54 Da. This venom peptide inhibits the response elicited by acetylcoline receptor molecules [27]. F7-3 peptide was clearly similar to αD-CTXs, and found to cause reversible inhibition of the acetylcoline induced response of the hα7 nicotinic acetylcoline receptors having an IC-50 value of 6.2μM, but it does not affect hα3β2 subunit [28].

### 2.7 E SUPERFAMILY

E-superfamily of conotoxins were discovered in the venom transcriptomes of Conus marmoreus and Conus Victoriae.
Mr104 from the venom of C. marmoreus, a 26 amino acids in length, with four cysteines and a bromotryptophan. Reports on this conotoxin functions, have not been identified yet. These conotoxins in the venom are having higher expression levels.

Im22.1 peptide was isolated from the venom of the vermivorous cone snail, *Conus imperialis*, having an amino acid sequence (NCKKNILRTYCSNKICGEATKNTNGELQCTMYCRCCANGCFRGQYIDWPNQQTNLLFC**)** with an average mass of 6568.47. Protein and cDNA analysis of the venom gland of the two specimens of *Conus imperilis* were predicted by 454 pyrosequencing and mass spectrometry. Its transcriptomic analysis revealed that 17 conotoxin gene superfamilies holding 5 superfamilies, two cysteine frameworks and highly expressed transcripts [29].

## 2.8 F SUPERFAMILY

F-superfamily of conotoxins were recently discovered from *Conus marmoreus (Fig. 6)* and *conus victoriae*. It has been defined as the only peptide signal sequence from each species.
This short Peptide product was identified (Mr105) from the putative pro-peptide sequence [30]. Similarly to E-conotoxin superfamily, functions for this superfamily have not been reported yet.

A peptide F_Vc1 isolated from *Conus victoria*, has an amino acid sequence of (KLMDTCAKANHYIADRWSTYRIEYLEDKGLYHRMLRELVPCLNNFLRTRQEAP) consists of about 53 residues with an average mass of 6442.43. *Conus victoria* is a molluscivorous cone snail.

## 2.9 G SUPERFAMILY

A peptide De13b isolated from *Conus delessertii* (Fig. 7), is considered as the only peptide identified, displays a type -XIII cysteine framework. It was reported that it also has a unique hydroxyl-lysine modification. It has 33 amino acid residues [(DCOTSCOTTCANG(Btr)ECC(hLy)GYOCVRQHCSGCNH(nh2)] with an average mass of 3595.72. It's a vermivorous cone snail species. It's novel structural and biological activity, expands the collection of disulfide-rich conotoxins that recognizes the mammalian nicotinic acetylcoline receptors [31]. It specifically inhibits to the voltage gated sodium and calcium channels, along with several nicotinic acetylcoline receptors [32].

## 2.10 G2 SUPERFAMILY

Conopeptide MiXXVIIA, a peptide similar to that of granulin promoting cell proliferation and inhibiting apoptosis. Its proliferative capacity on cholangiocytes, were observed at low micromolar concentrations and results were comparable to that of *O. viverinni granulin*. It's novel structural and biological activity expands the repertoire of disulfide-rich conotoxins recognizing the mammalian acetylcoline receptors. It specifically inhibits sodium and calcium voltage gated channels and a diverse range of nicotinic acetylcoline receptors. Anti-apoptotic activity is extremely rare for this toxin, however one conotoxin from *Conus californicus* recenty reported to possess a weak apoptotic activity at micromolar level in human lung cancer cells [33].

## 2.11 H SUPERFAMILY

The H-superfamily of conotoxins were isolated from *C.marmoreus* and *C.Victoriae*. H-superfamily of conotoxins identified so far, share the same cysteine residues similar to the O1, O2 and O3 superfamilies. Single sequence in this conopeptide encodes a cysteine free peptides.

A peptide H_Vc1.1 was isolated from *Conus Victoria*. This peptide belonging to M-superfamily has 24 amino acid residues (DVGSDRTSVELSKMLKGWQAEKGQ) with an average mass of 2649.96.

Conorfamide-Vc1(CNF-Vc1) was isolated from the cone snail *Conus Victoria* (Fig. 9). It represents an unusual conotoxin representing a cysteine-free, short motif common to known neuropeptides and hormones. One of these, conopeptide was the C-terminal RF-amide motif, commonly observed in neuropeptides. Mature venom peptides were isolated and characterized structurally and functionally. This peptide elicits an increased intracellular calcium level in DRG neurons. Whereas other neurons caused an increase in intracellular calcium levels in non-neuronal cells [34].

A peptide H_Vc1.2 isolated from *Conus victoria* has an amino acid sequence of KASAPKKFYVYPPV having 14 residues with a average mass of 1594.92. A peptide Vc7.2 isolated from *Conus victoria* has an amino acid sequence (NVNCGGVPCKFGCCREDRCREIDCD) consisting of about 25 residues with an average mass of 2786.10.

## 2.12 I1 SUPERFAMILY

I1 superfamily of conotoxins are significantly large and display a type XI cysteine pattern. It is the only peptide exhibiting excitatory activity. Conopeptides belonging to I1-superfamily define the important parameters for the post-translational i- to d-isomarization of amino acids. Ep11.1 peptide was isolated from *Conus episcopatus, and* has an amino acid sequence (GDWGMCSGIGQGCGQDSNCCGDMCCYGQICAMTFAACGP) comprising of about 39 residues with an average mass of 3894.34. This sequence was isolated directly from the precursor sequence, however, Some translational modifications are missing.

Three peptides such as, r11a, r11b and r11c induces a repetitive activity in frog's motor neurons. Epimerization of single amino acid causes attenuation of the two conotoxins r11a and r11b, however r11c acts on skeletal muscle.

## 2.13 I2 SUPERFAMILY

I2-Superfamily of conotoxins share the similar cysteine pattern as that of I1 Superfamily. These peptides are considered to be K+ channel modulators. I2-superfamily of conotoxins contain an unusual precursor structure, the encoded mature peptide directly follows the signal peptide, whereas the pro-peptide region located t the C-terminus of the precursor.

A peptide Betx was isolated from *Conus betulinus*It is a vermivorous cone snail. It was reported that BeTX acts on calcium activated potassium channels with an EC50 of 0.7nm and a hill coefficient of 0.88.

kappa-conotoxin isolated from *Conus betulinus* has four disulfide bonds, which does not have any homology towards other conotoxins. Kappa-BtX, is a specific modulator of voltage gated potassium (K+) channels. It also acts on the calcium (Ca2+) and voltage gated BK channels but does'nt have effect on single channel conductance. Kappa-BtX acts as a novel bio-toxin against BK channels [35].

## 2.14 I3 SUPERFAMILY

I3 conotoxin superfamily have similar cysteine framework as that of I1 and I2- superfamilies, but it displays a distinct signal peptide sequence. Ca11B isolated from *Conus caracteristicus* (Fig. 13), has 34 amino acid residues with an average mass of 3504.00. Peptides isolated and purified from *Conus characteristicus*, were designated as ca11a and ca11b. These peptides were found to have the I-superfamily cysteine patterns. These peptides were not able to display any post-translational modifications. These two peptide sequences were used to sequence cDNA encoding other I3-superfamily conotoxins.

G11.1 was isolated from *Conus geographus* and has an exquisite potential and high selectivity towards ion channels and serves as ideal source for drug leads in neuropharmacology. This venom peptide is capable of rapidly switching between venom types with different proteome profiles having specific responses towards predatory or for defense stimuli [37]. GXIA peptide has several stricking similarities towards numerous tarantula toxins, targeting sodium and potassium ion channels, because of the presence of the triple stranded beta-sheet. GXIA consisting of an amphipathic surface, a bled to be resided within the membrane and binds to the putative ion channel target via the voltage sensor domain [38].

## 2.15 INSULIN SUPERFAMILY

Con-Ins G1 isolated from *Conus geographus* has 209 amino acid residues with an average mass of 2306.96. This venom insulin has several structural similarities to the design of new insulin therapeutics. Fish hunting cone snail venom has the minimized ligands for the vertebrate insulin receptor. However, when these venom insulins were administered into zebrafish and mice, they significantly lowered the blood glucose level in streptozotocin induced diabetic mice. Insulins isolated from other cone snails such as from *Conus geographicus*, *Connus tulipa* (Fig. 14) and *Conus kinoshitai* exhibited diverse sequence, and had the capability of binding and activating the human insulin receptor and could be used as a novel drug for diabetic treatment.

Insulins isolated from *Conus geographicus* and *Conus tulipa*, comprises greater similarity to other fish insulins. These insulins are very much unique in their post-translational modifications, a characteristic of the conotoxins present in these cone snails [39].

### 2.16 J SUPERFAMILY

Isolation and characterisation of pI14a conotoxin from *Conus planorbis*, marks the identification of the J-superfamily. Structural determination of this conotoxin by NMR spectroscopy, revealed an helical pattern, which was unique among other conotoxins. This peptide when administered intracranially onto mice caused excitatory symptoms such as, shaking, rapid circling, barrel rolling and seizure formation. This peptide was reported to block thepotassium channels, and targets both the voltage-gated and ligand gated ion channels [40].

Ca14.3 isolated from *Conus caracteristicus* has 20 amino acid residues (IPVCSVLCNAGVDVPFCDCT) with an average mass of 2049.87. A high throughput transcriptomic sequencing analysis were used for isolating and analyzing the conotoxin transcripts from three vermivorous cone snails, such as *Conus caracteristicus*, *Conus generalis* and *Conus quercinus*.

### 2.17 K SUPERFAMILY

K-superfamily of conotoxins, im23a and im23b were isolated from the Conus imperialus venom, marks the discovery of new K-superfamily conotoxin and new cysteine framework XXIII (-C-C-C-CC-C-). Im23.3 isolated from *Conus imperialis* has an amino acid sequence consisting of about 39 residues with an average mass of 4452.12. Im23a peptide was found to be recombinantly expressed, and therefore adopts a helical hairpin fold. This fold had a series of acidic residues on its surface, serves as an important agent for calcium binding. Intracranial and intraveneous administrations in mice concluded that im23a and im23b significantly induces excitatory symptoms [41].

### 2.18 L SUPERFAMILY

L-superfamily of conotoxins were identified from the cDNA library of the *C.litteratus*. This peptide toxin displays a unique signal sequence. Cal14.1b induces cytotoxic effect on H1299 lung cancer cells, and decreased the presence of viability cells and does not increase Bax/Bcl-2 ration, because of the presence of Glutamate at the 15$^{th}$ residue. However, this is needed for Cal14.1a for inducing apoptosis. Cal14.2c was isolated from *Conus californicus.* Cal14.2c increased the cytokine IL-10 production of T-regulatory (CD3+CD4+Foxp3+) cells and however the cytokine IL-10 production in an 72hour post cultured cells were decreased [42].

It14a conotoxin with the globular disulphide arrangement, was able to inhibit an undetermined subtype of nAChR and also confirmed its analgesic activity in mouse hot-plate test. Analgesic reaction was observed against second phase pain in formalin induced inflammatory pain model and also in paw withdrawal threshold test of mechanical pain [35] (Ren et al., 2015). It inhibits calcium influx [Ca(2+) influx], ERK1/2 phosphorylation and c-fos/NOS expression [43].

### 2.19 M SUPERFAMILY

M-superfamily of conotoxins identified mostly display type III cysteine pattern. M-superfamily conotoxins have been divided into M1, M2, M3, M4, and M5 groups. These are classified according to their third intercysteine loop. All of these M-superfamily conotoxins share more precursor sequences. However, Conomarphins are slightly different again.

Conomarphine-Ac1 isolated from *Conus achatinus* serves as an analgesic agent. 10µM of conomarphin-Ac1 inhibited the NaV, KV, CaV, NMDA receptor channels [44]. GIIIA isolated from *Conus geographus* was reported to act on sodium channels and presumably interacts with site 1 on the Sodium channel pore. 10µM of this peptide rapidly blocks the A-fiber but not C-fiber [45]. Compounds participating in the action potential was found to be seen at the region of rat sciatic nerves. Synthetic µ-CnIIIC is an ideal blocker of the ion channels in the skeletal muscle and nerve cells and could be used as a myorelaxant [46]. Fainzilber et al., reported that PnIVB effectively blocks the tetrodotoxin resistant (TTX-R) sodium channels [47]. No effect was observed on mammalian system. Complete block of voltage gated Nav [48] (Sodium voltage gated channel) of Aplysia neurons at 80nM [49].

## 2.20 O1 SUPERFAMILY

O1-superfamily is the most thoroughly investigated group of conotoxins. Most of the O1-suerfamily conotoxins share type VI/VII cysteine framework. O1-superfamily of conotoxins exhibit relative diversity in their amino acid composition within each of the four intercysteine loops and at their N- and C- terminal diversity. O1-superfamily primary structure blocks inactivation of VGSCs. O1-superfamily conotoxin MrVIB is a drug lead in the development of novel analgesics.

Am 2766, isolated from *Conus amadis* was reported to inhibit the sodium channel currents in brain. A 10µM rapidly blocks the A-fiber compound action potential in mouse sciatic nerve [50]. Thus, mu-conotoxin PIIIA, was known to discriminate sodium channels [51]. A peptide PIIIA was isolated from *Conus purpurascens*. It belongs to M-superfamily of mu conotoxins having an amino acid sequence of about 23 residues [52].

The µ-conotoxin peptides similar to guanidinium alkaloids such as tetrodotoxin (TTX) and saxitoxin (STX), inhibiting sodium channels [53] (VGSCs) by blocking pores at NaV 1 [54]. It was reported that SIIIB interacts with site1 in the Sodium channel pore, as SIIIB completes with TIIIA and TIIIA in-turn completes with saxitoxin [55].

Voltage gated sodium channel inhibits current flow through the *Xenopus* oocytes expressing Na(v)1.2 and Na(v)1.4 respectively. Observations on Ts3.1 conotoxins led to the identification of mutator mechanism targeting the mature peptide domains in conopeptide genes [56]. The $hNa_v1.8$ peptide inhibitor TsIIIA provided a pharmacological probe for targeting the sodium channels and acting as a potential therapeutic agent for relieving pain [57].

## 2.21 O2 SUPERFAMILY

Am6.1 peptide was isolated from the venom of the cone snail *Conus amadis.* This peptide belongs to O2 superfamily comprising of an amino acid sequence with 28 residues. It has an average mass of 3248.06. However, Vijayasarathy et al. reported that unmodified and partially modified sequences were also found to be present in the venom. Two cysteine frameworks existing within the O2 superfamily, consisting of three precursors (Mi035–037) with a Type XV (C-C-CC-C-C-C-C) framework that was identified using MS/MS after enzymatic digestion [58].

## 2.22 O3 SUPERFAMILY

O3-Superfamily conotoxins share similar cysteine patterns observed in O1- and O2- superfamily conotoxins. Only one peptide called as the “Bromosleeper” conotoxin under the O3-superfamily has been fully characterized. Symptomatology is similar but not identical to that of conantokins, but these are inhibitors of NMDA receptors. Peptides purified and characterized from the *C. radiatus* induces lethargy, drowsiness and sleep in mice. Addition to bromotryptophan, this bromosleeper peptide has other post-translational modifications. G27 peptide among the O3-superfamily have unusual cysteine framework (C-C-CCC-C-C-C).

A peptide T16.1 isolated from *Conus tulipa* belonging to O3 superfamily of class II cysteine has an amino acid sequence comprising of about 24 residues with an average mass of 2961.58.

Conantokins (NMDA antagonists) are conopeptides isolated from the B1 superfamily, dominating the transcriptomics and proteomics of *Conus tulipa* venom. *Conus tulipa* venom comprises of both paralytic and non-paralytic (conantokins, con-ikot-ikots, conopressins) conopeptides confirming that these non-paralytic conotoxins are used for the hunting strategy of *Conus tulipa* cone snail [59].

### 2.23 P- SUPERFAMILY

Prototypical P-superfamily conotoxin, TxIXA was isolated from the venom of the *C.textile*, eliciting "spasmodic" activities in mice. TxIXA peptide binds in a non-competitive fashion. Few of the P-superfamily members have been identified. One peptide gm9a, shares a similar sequence and activity to that of TxIXA. It was reported that some unlikely peptide regions involved in receptor binding was also found.

BeTXIIb isolated from *Conus betulinus* has 27 amino acid residues with an average mass of 2644.83. The biological activity of the venom peptide isolated from *Conus betulinus* showed that these are a set of small peptides with a high cysteine content [60].

GmIXA isolated from *Conus gloriamaris* (Fig. 18) has 28 amino acid sequence residues with an average mass of 2798.03. A chemically synthesized peptide, gm9a was found to elicit the same symptomatology, which has been previously described for native folded peptides such as, tx9a and gm9a. However, gamma-carboxyglutamate (Gla) residues such as, serine, alanine, glycine etc, which are found in tx9a aren't crucial for biological activity [61].

### 2.24 Q SUPERFAMILY

Q-superfamily of conotoxins were discovered recently from *C.flavidus.* Group of conotoxin like venom gland transcripts were identified, however, its signal peptide sequence did not match the previous superfamily. Peptide precursors identified from Q-superfamily precursors were confirmed by MS/MS matching. This Q-superfamily was also identified in C. quercinus and C. caracteristicus. A new Q-superfamily conotoxin, qc16a have a ribbon-type disulphide connectivity and in solution this peptide adopts a simple beta-turn motif. This peptide when administed intra-cranially caused depression in mice. Electrophysiological analysis proved that, qc16a had no strong effect on the whole-cell currents of neurons [62]. Fla16.1 isolated from *Conus flavidus* has 13 amino acid residues (DCQPCGHDVCCPP) with an average mass of 1369.51.

### 2.25 R SUPERFAMILY

VilXIVA isolated from *Conus villepinil* has an amino acid sequence 27 residues with an average mass of 2873.35. *Conus villepinil* is a *vermivorous* cone snail. It was reported that VilXIVA was a part of kappa family playing an important role in blocking potassium channel [63]. It exhibits a cysteine framework of CCCC connectivities.
F14 conotoxins were isolated from the cone snails, *Conus anabathrum* and *Conus villepinii*. Transcriptomic analysis gave the full-length sequence of the precursors flf14a and flf14b isolated from *Conus anabathrum.* Analysis revealed that this peptide has a unique signal sequence. Using the signal sequence as primer, several additional undescribed toxins of the R-superfamily conotoxins were cloned. However, these peptides are called as Proline-rich motifs (PRMs), which are needed for protein-protein interactions.

### 2.26 S SUPERFAMILY

First S-superfamily of conotoxins is GVIIIA conopeptide. This peptide was identified from the venom of the C.geographus, capable of inhibiting the serotonin-activated currents. Phramacological characterization reported that this peptide is a competitive agonist [3H]-zacopride in HEK293 cells stably expressing 5-HT3 receptors. This peptide was reported to inhibit the neuromuscular nAChRs. It also had activity over several different

neuronal subtypes. New peptide identified under the S-superfamily, ca8a isolated from the venom of the *C.caracteristicus*. This peptides cDNA sequence allowed the cloning of several other S-superfamily conotoxins. GVIIIB isolated from *Conus geographus* belongs to the S superfamily of α-conotoxins with an average mass of 4464.89. It was reported that GVIIIB, 100-fold selective for the α9α10 nicotinic acetylcoline receptor (nAChR) compared to other nAChR subtypes such as the alpha1beta1δε, 5-HT3 serotonin receptor, α3β2β3, β α6/α3β3β3, α3β4, α4β2 and α7[64]. This peptide targets the 5-HT3 receptor and αS-GVIIIB conotoxins block the α9α10 nAChR[65]. RVIIIA caused paralysis in mice and fish causing irreversible block on the neuromuscular nicotinic acetylcoline receptors (nAChR) [66].

## 2.27 T SUPERFAMILY

T-superfamily of conotoxins comprises two conopeptide groups such as, the Tau- conotoxin and chi-conotoxin group. Am1.1 was isolated from the cone snail, *Conus amadis* belonging to T-superfamily, consisting of 15 amino acid residues with an average mass of 1485.55. Chi-conotoxins has a unique ability of non-competitively impeding the noradrenaline transporter. These Chi-conotoxins have an unusual cysteine-stabilised scaffolds holding the gamma-turn for interacting with the noradrenaline transporter [67].

Lt5d isolated from *Conus litteratus* consists of an amino acid sequence of DCCPAKLLCCNP, consisting of 12 residues with an average mass of 1274.49. This peptide blocks terodotoxin sensitive (TTX-S) Sodium Na channels [68].

T-superfamily conotoxins are examples of conotoxin superfamily, for which little precursor sequences have been reported. However, only a little is known in terms of their pharmacological properties. T-superfamily of conotoxins were subdivided into three groups based on their cysteine framework. T-superfamily conotoxin, TxXIIIA is a unique peptide derived from *C.textile*, contains an extra cys residues. This peptide venom has a homodimer, however no reports on its disulphide bonding pattern is available. X-framework conotoxins from the T-superfamily were first discovered in *C.marmoreus*. CMrX isolated from *C. marmoreus* causes paralysis and death in mice upon IC injection.

## 2.28 Y SUPERFAMILY

**Y-superfamily of conotoxin, ca17a was isolated from the venom of *C.caracteristicus*. Its precursor sequences were determined using the RACE-PCR of the C.caracteristicus venom gland. This peptide has a cysteine framework of XVII cysteine framework (C-C-CC-C-CC-C).**

Conopeptides isolated from three vermivorous cone snails namely, *Conus caracteristicus, Conus generalis* (Fig. 20) and *Conus quercinus*. High throughput transcriptomic sequencing was performed for analyzing and extracting the venoms from these cone snails [69]
Ca17.1 isolated from *Conus caracteristicus* has 34 amino acid residues with an average mass of 3523.95. Peptide ca16a, containing eight cysteine residues were isolated, purified and cloned from *Conus caracteristicus*. It is a hydrophobic peptide having 34 amino acid residues comprising of 4 acidic and 4 basic residues. This peptide is rich in polar amino acids (Glycine, Serine, Threonine, hydroxylated proline). Its sequence does not have any homology to other conotoxins and represents a new class of conotoxin family [70].

## 2.29 CONODIPHINE SUPERFAMILY

Conodipine-M is a 13.6 KDa peptide isolated from the venom of Conus magus. This peptide sequence was partially characterized and are quite different from other conotoxins. It is made up of two polypeptide chains, an α- and a β-chain. This peptide showed phospholipase-A2 activity and also need Ca2+ as a cofactor.

Phospholopase-A2+ activity was reported in animal venoms, as well as in mammalian tissues and bacteria. These phospholipases catalyze the hydrolysis of ester bond at the sn-2 position of 1,2-diacyl-sn-phosphoglycerides. In addition to phospholipase activity, it also performs neurotoxicity.
Structure of conodipine genes were discovered from the venom gland of transcriptome C.victoriae. Precursors of this peptide contains a α-chain, a propeptide sequence and a β-chain. This conodipines also constitute multiple conotoxin superfamily.

Conodiphine-P5 beta chain was isolated from the venom of the vermivorous cone snail, *Conus purpurascens* having an amino acid sequence of 47 residues with an average mass of 5173.63.

Three conopeptides belonging to Conodiphine family namely, conodiphine-P1, conodiphine-P2 and conodiphine–P3 were isolated from the cone snail, *Conus purpurascens*. These conopeptides were then purified and their structures were determined using MS-spectrometric methods. Phospholipases A (PLAs) is found in the venom of these peptides and these phospholipases are composed of two chains termed as alpha and beta subunits. These phospholipases-A in general are small ubiquitous enzymes catalyzing the hydrolysis of sn-2 ester bond. Conodiphines have medicinal properties such as inhibiting cancer cell proliferation and preventing bacterial and viral infections [71]. Conodiphine subunits: Conodhipine-P1-3 contains Asp/His dyad, catalytic domain residues being expressed in a mixture of proline hydroxylated isoforms.

## 3. CYSTEIN FRAMEWORKS

### 3.1. CYS FRAMEWORK I

AuIB conotoxin has a Cysteine framework of CC-CC connectivity. This peptide inhibits the N-type calcium (Ca2+) channels in rat DRG neurons [72].

Sodium channel blocking peptides namely muPnIVA and muPnIVB were isolated from the molluscivorous cone snail, *Conus pennaceus*. Amino acid sequences of these peptides were complicated by the presence of reduction and pyridylethylation occuring at the N-terminal cysteine residue and giving a post translationally modified derivative using the reverse-phase chromatography. The sequences of these conotoxins were determined by using Edman degradation and mass-spectrometric techniques. These conotoxins block the sodium channels in molluscan neurons, but had no effect on bovine chromaffin cells in rat brain synaptosomes.

### 3.2. CYS FRAMEWORK II

S1.2 conotoxin has a cysteine framework of CCC-C-C-C connectivity. An acetylcholine receptor blocker, α-conotoxin SII have three disulfide bonds (instead of two and a free C-terminus [73].

Two toxins im23a and im23b has a cysteine framework of C-C-C-CC-C connectivity's. Disulfide connectivity of im23a were mapped by using chemical mapping and NMR structure calculations, both of which establish a I-II, III-IV, V-VI disulfide connectivity's. Solution structure of im23a reveals that it has a helical hairpin fold with a cluster of acidic residues on its surface, capable of binding to calcium. When this peptide was administered intra-cranially and intra-venously onto mice induced excitatory symptoms.

### 3.3. CYS FRAMEWORK III

EI conotoxin (Fig. 21) from *Conus ermineus*, has an amino acid sequence (RDOCCYHPTCNMSNPQIC) with an average mass of 2093.36. It has a cysteine framework of CC-C-C-CC connectivity's.

Sodium channel blocking peptides, muPnIVA and muPnIVB were isolated from *Conus pennaceus.* These conotoxins block the sodium channels in molluscan neurons, and does not produce any effect on sodium channels

present in bovine chromaffin cells or in rat brain synaptosomes [74]. These structurally novel mu-conotoxins, acts as a new source for functional studies on sodium channels.

### 3.4. CYS FRAMEWORK IV

Conopeptides, muPnIVA and muPnIVB were isolated from the cone snail, *Conus pennaceus*. The former peptide have an amino acid sequence of CCKYGWTCLLGCSPCGC and later has a sequence of CCKYGWTCWLGCSPCGC. They exhibit a new disulfide framework of CC-C-C-C-C connectivity's, and share a –CC- -CC- consenses signal sequence. Conopeptide PnIVB causes block on the sodium channels in *Lynmnaea* neurons and also in *molluscan* neurons. It does not block the sodium channels present in bovine chromaffin cells. However, these mu-conotoxins target sodium channels with low affinity for tetrodotoxin [75].

### 3.5. CYS FRAMEWORK IX

Turritoxin, pal19a, has an amino acid sequence with 34 residues, consisting of 6 cysteine disulfide residues with a framework IX consisting of C-C-C-C-C-C-C connectivities [76].
Its induction on normal mice, however specifically distinguished it from the mutant, characterized it to be the spasmodic mice. It has an amino acid sequence comprising of about 27 residues, holding two gamma-carboxyglutamate (Gla) residues [77].

### 3.6. CYS FRAMEWORK V

Conopeptides p5a (Fig. 22), tx5a, au5a belonging to T-superfamily were identified from the cone snail venom duct. They have a novel cysteine framework of - -CC- -CC- connectivities. These peptides have amino acid sequences ranging from an order of 11 to 17 residues. However, they exhibited a varying degree of posttranslational modifications. Only one among these three peptides were active on mice, whereas the rest didn't have any effect on fish [78].

### 3.7. CYS FRAMEWORK VI/VII

Conopeptide Ω-CgTX was isolated from *Conus geographus*. These peptides have an amino acid sequence with 27 residues with preponderance of hydroxylated amino acids. It has a cysteine framework of C-C-CC-C-C connectivities with three disulfide bridges. This conopeptide is capable of inhibiting calcium entry ($Ca^{2+}$), providing a potential for exploring the presynaptic terminal on the vertebrate neurons [79].
Conopeptides with a novel cysteine framework VI/VII, was isolated from *Conus victoriae*. Having cysteine framework with disulfide connectivities occurring in the cone snail between C1-C4/C2-C5/C3-C6. This peptide has close association with cysteine knot (ICK) fold inhibitor [80].

### 3.8. CYS FRAMEWORK VIII

GVIIIA has 41 amino acid residues with a cysteine framework pattern of C-C-C-C-C-C-C-C-C-C-C connectivity's. Final sigma conotoxin, consisting of brominated tryptophan residue with endogenous ligand for its tryptophan derivative hydroxylated 5-HT3 receptor. This final sigma conotoxin inactivates the 5-RHT3 receptor through competitive antagonism. Serotonin receptors are molecular targets for natural polypeptide neurotoxins. This peptide is a excitatory serotonin-gated ion channel specifically acting on specific receptor ion channels mammalian nervous system [81].

### 3.9. CYS FRAMEWORK X

It has a unique disulfide pattern exhibiting a unique "ribbon" conformation important for their biological activity. These peptides have disulfide connectivities of C-C-CC-C-C, and also inhibits the cysteine knot and CC-CC motifs.

Disulfide connectivities comprising C(1)-C(4), C(2)-C(3) patterns were identified by reverse-phase high performance liquid chromatography. Cysteine residues found are comparable to that of alpha-conotoxins. These peptides were named as CMrVIA (Fig. 23), CMrVIB and CMrX. Conotoxins CMrVIA and CMrX exhibiting different disulfide bonding patterns, naming them as lambda conotoxins. These two toxins, when administered intra-cerebroventricularly onto mice caused several biological effects. CMrVIA conopeptide induced seizures whereas CMrx caused flaccid paralysis [82].

### 3.10 CYS FRAMEWORK XII

Conopeptides, Gla-TxX and Gla-TxXI were isolated from *Conus textile*. These peptides have a synthetic 13 amino acid residues with a C-C-C-C-CC-C-C-C connectivity's. C-terminal post-peptides found in these conotoxins, indicates the presence of gamma-carboxylation recognition site. This gamma-glutamyl carboxylase catalyzes the conversion of glutamyl residues to gamma-carboxyglutamate. However, the precursors of these peptides are mostly vertebrate proteins involved in various biological processes such as blood coagulation, bone mineralization, and invertebrate ion channel blockers [83].

### 3.11. CYS FRAMEWORK XIII

Peptide de13a, was isolated from the cone snail, *Conus delessertii*. It has a high amino acids sequence, which is post-translationally modified containing the 6-bromotryptophan and a non-standard amino acid 5-hydroxylysine. It has a cysteine framework of C-C-C-CC-C-C-C connectivity's. However, this connectivity differs from the ones designated in the cysteine frameworks of 13 or XIII [84].

### 3.12. CYS FRAMEWORK XIV

Conopeptides flf14a and flf14c were isolated from *Conus floridanus floridensis* and another conopeptide vil14a was isolated from *Conus villipinii*. These peptides represent four-cysteine, with cysteine framework patterns of C-C-C-C connectivity's. Conopeptide vil14a consist of Lys/Tyr dyad, its conserved feature is to block the K+ channel. These peptides have a highly stable cysteine peptide scaffolds which serves as a part for their neurochemical strategy to capture their prey [85].

Peptide di16a, was isolated, purified and characterized from the cone snail, *Conus distans*. These excitatory peptides have five of its amino acids to be modified, one was modified on the basis of gamma-carboxygluatamate (Gla) and the other four were modified on the basis of hydroxyproline (Hyp) and exhibiting a C-C-C-CCC-C-C-C-C connectivity. These peptides when injected caused hyperexcitable changes in 3 weeks old mice at lower doses and with higher concentrations lethargy was observed [86].

### 3.14. CYS FRAMEWORK XVI

Expressed sequence tags (ESTs) of about 897 sequences derived from *Conus litteratus* were analyzed to define the diversity and evolutionary aspects of conotoxins. These peptides have a cysteine framework of C-C-CC connectivity. Almost, half of these expressed sequence tags were coding sequences of conotoxins. Divergence within the superfamily were found to be increased from N terminal to C terminal in the open reading frame and also mature peptide scaffolds exists in this conotoxin gene superfamily [87].

### 3.15. CYS FRAMEWORK XVII

Conotoxin ca16a was isolated from *Conus caracteristicus*. It was then purified and sequenced yeilding a hydrophobic peptide with 34 residues, having four acidic and four basic residues.

Its residues are rich in polar amino acids (Gly, Ser, Thr and hydroxylated proline). Its cysteine residues comprise a framework XVI with C-C-CC-C-CC-C connectivity. This conotoxin is less toxic and possess acetylcoline receptor activity and potassium current effect [88].

### 3.16. CYS FRAMEWORK XVIII

Conopeptides BeTXla, BeTXlb, BeTXlla and BeTXllb were isolated from *Conus betulinus*. These peptides were then purified using gel-filteration followed by HPLC and finally sequenced by using the ABI model 491 sequencer. BeTXla and BeTXlb conotoxins has 14 and 15 amino acid residues, while BeTXlla and BeTXllb has 27 and 30 amino acid residues, respectively. These low molecular weight conopepti des has a high cysteine content. It has a cysteine framework of C-C-CC-CC connectivity. These peptides have low toxicity and possess acetylcoline receptor activity and potassium current effect [89].

### 3.17. CYS FRAMEWORK XX

Peptides αD-conotoxin VxXllA, VxXIIB and VxXIIC were isolated from *Conus vexillum*. Exhibits a framework pattern of C-CC-C-CC-C-C-C-C connectivities. These αD conotoxins are inhibitors of nicotinic acetylcoline receptors (nAChRs). The cDNA analysis of these peptides isolated from Clade XII species such as *Conide Conus vexillum*, *Conus capitaneus*, *Conus mustelinus* and *Conus miles* revealed a greater heterogeneity and its phylogenetic analysis displays a EMM or AVV signal peptide sequence motif [90].

### 3.18. CYS FRAMEWORK XXI

Conotoxin p21a was isolated from *Conus purpurascens*. Its sequence was determined using Edman degradation yeilding complete sequences. This analysis showed that p21a have a unique, 10-cystein/5-disulfide 7-loop framework with extended 10-residue N-terminus and a 5-residue C-terminal tails with a cysteine framework pattern of CC-C-C-C-CC-C-C-C connectivity. Conopeptide p21a has a 48% homology for con-ikot-ikot conopeptide isolated from *Conus striatus* and it does not form any dimer [91].

### 3.19. CYS FRAMEWORK XXII

Conopeptides isolated from cone snail, *Conus californicus*, using its DNA and protein analysis, molecular diversity of this conotoxin detects correspondingly a large number of conotoxin types exhibiting a cysteine framework of C-C-C-C-C-C-C-C connectivity. All of these peptides were analyzed using mass spectrometry and this served to identify peptides corresponding to a number of cDNAs, all differing in their degree of post-translational modification [92].

### 3.20. CYS FRAMEWORK XXIII

Two conopeptides, im23a and im23b were isolated from the cone snail *Conus imperialis*. These toxins have a cysteine framework of C-C-C-CC-C connectivity. Disulfide connectivity of im23a (Fig. 24) using NMR structural calculations yeilded a I-II, III-IV, V-VI disulfide bridges. However, the solution structure revealed that im23a adopts a hairpin fold. This peptide has a cluster of acidic residues found at its surface, capable of binding to calcium. When administered intra-cranially into mice caused excitatory symptoms [93].

### 3.21. CYS FRAMEWORK XXIV

αB-conotoxin, VxXXIVA was isolated from *Conus vexillum*. Mature peptides consists of 40-residues, with four cysteine residues. Its mechanism of action revealed that it acts as a nicotinic acetylcoline receptor antagonist with greatest potential towards α9α10 subtype. Circular dichroism (CD) showed that this peptide was unstructured in buffer, but adopted a partially helical conformation in aqueous trifluoroethanol (TFE) solution. It has a disulfide framework of C-CC-C connectivity. Thus, α9α10 nicotinic acetylcoline receptor could be used in the development of analgesics and cancer chemotherapeutics.

Electrophysiological analysis using superior cervical ganglion (SCG) neurons indicated that RsXXIVA inhibits calcium channel (CaV2.2) in a dose-dependent manner. It has anti-nociceptive effect.

### 3.22. CYS FRAMEWORK XXV

Conotoxin as25a, a 23 amino acid peptide was isolated from *Conus cancellatus*. This peptide was characterized and found to have a sequence pattern of CX1CX2CX8CX1CCX5. Intracranial administration of this peptide into mice provoked paralysis on its hind limb and caused death with a single dose of 240pmol. Post-translational variation was found to have two hydroxyproline residues [95].

### 3.23. CYS FRAMEWORK XXVI

Conopeptide RsXXIVA was isolated from *Conus regularis*. Primary structure of this peptide was determined using mass spectrometry and confirmed using automated Edman degradation. It has an amino acid sequence with cysteine residues of C-C-C-C-CC-CC connectivity. Electrophysiological analysis was done on the superior cervical ganglion (SCG) neurons indicating that this peptide, could inhibit calcium (CaV2.2) channels in a dose-dependent manner. It was reported to contain anti-nociceptive effect and also had analgesic effect [96].

### 3.24. CYS FRAMEWORK XXVIII

Conotoxin, Lt28.1 belonging to D-superfamily was characterized. Recombinant Lt28.1 targets the α9α10 nicotinic acetylcoline receptor subtypes [97].
Conopeptides belonging to D-superfamily consists of 10 cysteine residues with a C-CC-C-CC-C-C-C-C connectivities. These peptides are known to specifically act on the nicotinic acetylcoline receptor. Several of these precursors with undescribed cysteine arrangements of C-C-C-CC-C-C-C-C connectivity found from RACE [98].

### 3.25. CYS FRAMEWORK XXX

Conopeptides belonging to O1-superfamily was isolated from cone snail *Conus californicus*. This peptide was purified by using reverse phase high performance liquid chromatography (RP-HPLC) and sequences were constructed using Edman degradation. However, complete sequences were given by RACE. This peptide has an amino acid sequence of 32 residues containing eight cysteine residues with C-C-CCC-C-C-C-CC connectivities. This conotoxin inhibited the growth of *Mycobacterium tuberculosis* (Mtb). when tested against pathogen refere nce strain (H37Rv) and multidrug resistance strains, this peptide showed growth inhibitory effect with a minimum inhibitory concentration (MIC) in the range of 3.52 to 0.22µM, similar effects to drugs used in clinics [99].

### 3.26. CYS FRAMEWORK XXXII

A novel conotoxin Mo3964 (Fig. 25), has a cysteine framework connectivity C-CC-C-C-C. Its tertiary structure fold has not yet been found in any of the Cone snail venom peptides. Ensemble structures of this peptide was found at the backbone with 87% and 13% of its dihedral angles lying on the most favored and additionally allowed regions in the Ramachandran plot. Mo3964 conopeptide decreased the outward potassium channels in rat dorsal root ganglion neurons [100].

## 4. PHARMACOLOGICAL FAMILY

**Conotoxins are a group of neurotoxic peptides isolated from cone snails**. However, it was reported that many of these conopeptides have capability to modulate the activity of various ion channels. First conopeptide was isolated from the cone snail venom and described at the peptide level.

Pharmacological families were classified based on their target receptor specificity of the conopeptide and denoted by a Greek letter. Totally, twelve pharmacological families have been designated (www.conoserver.org) yet only 167 conopeptides, have been assigned to a pharmacological family. The main reason for this low number is that, the pharmacological family can only be determined with functional experiments and not through the use of bioinformatics methods.

### 4.1 ALPHA CONOTOXIN

Alpha-Conotoxins (α-CgTxs) are a family of cysteine rich peptides. These peptides act as competitive antagonists to acetylcholine receptor (nAChR) [101]. Conopeptide AuIB was isolated from *Conus aulicus*. It is a non-native ‘‘ribbon’’ disulfide isomer exhibiting enhanced activity at the nAChR [102]. It has 10-fold potential compared to α-AuIB, having a 25-fold lower activity.

Alpha conotoxins [R9A]GI have no effect for the two acetylcoline binding sites on Torpedo receptors, but its site-specificity was apparently abolished. This is the very first characterized toxin from *conus* venom [104]. MIIIJ isolated from *conus magus* induced paralysis in gold fish. It was also predicted that MIIIJ is from M-Superfamily but lacks precursor data [105]. MrIC selectively activates the α7nAChR, despite being modulated by type-II allosteric modulators [106]. Jin et al. 2014 stated that this peptide is coming from a precursor named Mr1.7 [107]. This conotoxin is a co-agonist for nicotinic acetylcoline receptor (nAChR). PeIA conopeptide was isolated from *Conus pergrandis*. PeIA was more potent on α6/α3β2β3 than in α3β2 receptors [108]. Alpha Conotoxin MII (Fig. 26), is a selective ligand discriminating wide variety of nAChR subtypes, but fails to act on the α3 and α6 subtypes.

### 4.2. CHI CONOPEPTIDE

Novel conotoxin MrIA, (Fig. 27) was isolated from the venom of the cone snail, *Conus marmoreus*. MrIA belonging to T-superfamily has an average mass of about 1408.64. It selectively targets the norepinephrine transporter and it did not have any effect on dopamine or serotonin uptake through DAT and SERT transporters respectively. It also inhibits the binding of Norepinephrin transporter inhibitors. However, higher sodium (Na+) concentrations improved the inhibitory activity. It was reported that G6A mutation causes a significant change in its structural perturbation [109]. MII conopeptide blocked the human α7 and α1β1γδ receptor subtypes.

### 4.3. DELTA CONOTOXIN

Delta-conotoxins are capable of slowly inactivating the voltage gated sodium [Na(V)] channels. These delta conotoxins interaction and mechanism of channel modulations are not known yet. Delta-conotoxin SVIE was isolated from *Conus striatus* and was reported to have interaction with the domain-4 of ion channels [110].

Mo3964 conopeptide (Fig. 28) from *Conus monile* defines a new Cysteine framework. Mo3964 was found to reduce the outward currents during the opening of Voltage gated K+channels in DRG neurons (Kancheria et al.,2015). However, the leftward shift occuring in an average normalized conductance on the sodium channel currents were observed in Mo3964 conopeptide (Kancherla et al., 2015). Conopeptide SuVIA isolated from *conus sulturatus* has an amino acid sequence of about 27 peptide residues. δ-conotoxins are a potent ichtyotoxins, that has the capability to enhance sodium channel functions [111].

### 4.4. EPSILON CONOTOXIN

Conotoxin TxVIA was isolated from the cone snail, *Conus textile*. TxVIA (Fig. 29) belonging to T-Superfamily has a average mass of about 1931.74. Eight different T-superfamilies share a consensus signal sequence with the cysteine residues of (- -CC- -CC-). Epsilon-conotoxins act at the presynaptic sites, blocking the calcium channels. Intra-cranial administration of this peptide, into mice caused hyperactivity and drooping of dorsal fins in fish. Peptides of this superfamily exhibits a varying degree of post-translational modifications [112].

The disulfide bonding had various post translational modification processes such as the bromination, hydroxylation, and glycosylation that could target the presynaptic $Ca^{2+}$ channels. This conotoxin selectively

reduced the neurotransmitter release at *Aplysia* cholinergic synapse by reducing the presynaptic influx of $Ca^{2+}$ in a slow and reversible fashion [113].

### 4.5. GAMMA CONOTOXIN

TxVIA conotoxin was isolated from *Conus textile*. Gamma-conotoxins may act on voltage-gated channels. This peptide showed toxic effect on the freshwater snail *Pomacea paludosa* after intramuscular injection, but it had no effect intracerebral administration in mice.

Eight different T-superfamily share a consensus signal sequence, and conserved arrangement of cysteine residues (- -CC- -CC-). Gamma-conotoxins may act on voltage-gated channels [114]. Intramuscular injection elicits toxic effects on *Pomacea paludosa*. Different peptides of the superfamily has diffent levels of post-translational modification (Craig et al.,1999). Post translational modifications including bromination, hydroxylation, and glycosylation, targeting the presynaptic $Ca^{2+}$ channels [115]. Conotoxin isolated from *Conus textile* consist of unusual post translational modifications effectively reducing the presynaptic calcium influx. This conotoxin reduced the presynaptic calcium influx ($Ca^{2+}$ ) by decreasing the neurotransmitter release [116].

### 4.6. IOTA CONOTOXIN

Iota-conotoxins binds to voltage-gated sodium channels acting as agonists by shifting the voltage-dependence to more hyperpolarized level. However, when these iota conotoxins are administrated intra-corporeal into frog, caused excitatory symptoms to take place at the cutaneous pectoris muscle.

Conotoxin LtIIIA isolated from *Conus litteratus* has an average mass of 1964.16. LtIIIA [117] acts as an agonist of sodium channels. Effects of BF evaluated in embryo-larval zebrafish caused the hatching rate to accelerate in an concentration-dependent way. Mutation on f44>F caused loss in activity of the amphibian axons [118]. Elevation of reactive oxygen species levels, depletion of mitochondrial membrane potential and intracellular glutathione were observed during the lead exposure of this conotoxin.

### 4.7. KAPPA CONOTOXIN

A novel conopeptide RIIIJ was isolated from the cone snail, *Conus radiates*. RIIIJ has an average mass of about 2807.29. These peptides provide an essential pharmacological role in systematically targeting the specific heteromeric voltage gated potassium channel complexes [119]. RIIIJ blocks heteromeric voltage gated potassium channels containing the Kv1.2 stigma subunit and other subunits from the Kv1 family (Kv1.x).
SIVA conopeptide, belonging to kappa conotoxin, upon intra-peritonial administration into fish, caused rapid swimming followed by spastic. Kappa conotoxins inhibit the voltage-gated potassium channels.

### 4.8. MU CONOTOXIN

Mu-conotoxins blocks the voltage-gated sodium channels (Nav). Conotoxin CIIIA, was isolated from the cone snail, *Conus catas.* This peptide selectively blocks the tetrodotoxin resistant (TTX-R) sodium channels [120]. CnIIIA conopeptide alone caused paralysis. 10μm of this peptide conotoxin blocks the A-fiber but not C-fiber action potentials in mouse [121]. Toxin embryonic mRNA expression was initiated before the differentiation of the venom gland [122]. Embryonic α-conotoxins has the same three-dimensional structure as that of the adult conotoxin.
Neurotoxic cone snail peptide μ-GIIIA (Fig. 30), specifically blocks the skeletal muscle voltage-gated sodium ($Na_V1.4$) channels. On the other hand, a broader target range of the μ-conopeptides also antagonize the subtypes of voltage-gated potassium channels [123]. KIIIA peptide acts by blocking the tetrodotoxin resistant (TTX-R) sodium channels. It also blocks the C-fiber CAPs. This conotoxin reduces neurotransmitter release [124].

Alpha –Conotoxin EpI was isolated from *Conus episcopatus.* It appears to be an extremely potent and selective inhibitor for α3β2 and α3β4 receptors [125]. Conotoxin SIIIA isolated from Conus striatus causes A-fiber compound action potentials in mouse sciatic nerve. Slowly blocks C-fiber CAPs [126].

### 4.9. OMEGA CONOTOXIN

Omega-conotoxins act at the presynaptic sites block the voltage-gated calcium channels. Contryphan-Am isolated from *Conus amadis* has an average mass of about 976.09. Contryphan-Am inhibits voltage gated Calcium channels [127]. μ-conopeptides with a broader targeting range, selectively antagonize the select subtypes [128].

Contryphan-M isolated from *Conus marmoreous* has an average mass of about 1472.52. Contryphan-M is a blocker of calcium channels. Eu1.6 belonging to A Superfamily and was isolated from *Conus eburneous*. Eu1.6 exhibits potent analgesic activity in rat and inhibits HVA calcium channels in rodent DRG neurons [129].

## 5. CONOPEPTIDE CLASS

### 5.1. CON-IKOT-IKOT

Con-ikot-ikot was identified from the venom of the cone snail, *C. Striatus*. It is a unique conotoxin eliciting effects on the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid receptors, thereby inhibiting channel desensitisation. This conotoxin precursor encoded a large number of conotoxins having around 86 amino acid residues. This conopeptide exists as covalent homodimer with three inter-subunit disulphides. This homodimeric peptide toxin fills the gap in between the amino terminal- and ligand binding domains of the AMPA receptor. This proves the insight of this peptide into receptor desensitization and paves the way for the development of new therapeutic agent.

Con-ikot-ikot was isolated from *Conus striatus*. It has 86 amino acid residues with an average mass of 9432.69. The first 27 amino-acids have been sequenced by Edman degradation. X-ray crystallographic data indicates that it acts as an dimeric complex. Agonist is required for toxin-receptor binding. Toxin spans the receptor gating ring [130]. *Conus striatus* toxins acts as a positive allosteric modulator and as orthosteric agonists [131]. Analysis of con-ikot-ikot to hippocampal slices caused rapid increase in resting AMPAR-mediated current leading to neuronal death. Conotoxin p21a, has a unique 10cystein, 5disulfide and does not form a dimer [132].

### 5.2. CONOTOKIN-G

Conantokins were isolated from the venom of the pisivorous cone snail and has 18 amino acid residues with an average mass of about 2264.21[133]. Conantokin-G is active against the NMDA glutamate receptors, and acts as an NR2B-selective competitive antagonist [134].

Conantokin G (Con G) acts as an antagonist to N-methyl-d-aspartate (NMDA) receptors. GVIA is a ω-conotoxin, specific inhibitor of Cav 2.2, a voltage-gated Calcium channel. Conantokin-G and conantokin-T are naturally occurring peptide components [135]. Con-G acts as a neuroprotective agent, with an excellent therapeutic potential to combat ischemic/excitatory brain injury [136].

### 5.3. CONANOTOKIN-L

Novel conopeptide, conanotokin-L was isolated from the venom of *Conus lynceus*. This peptide has 20 amino acid residues. Conantokin belonging to B1-Superfamily has an average mass of about 2207.26. Conantokin-L is active against the NMDA glutamate receptors. C-terminal sequences of conantokin-R and conantokin-L are the main components responsible for anticonvulsant activity [137].

### 5.4. CONANTOKIN- T

Conantokins are 21-amino acid residues, capable of inducing sleep-like symptoms in young mice. This peptide was purified and its amino acid sequence were determined using chemical synthesis. Conantokin-T causes inhibition of N-methyl-D-aspartate (NMDA) receptor [138]. Two cysteine-rich antibacterial peptides such as, turgencin A and turgencin B were isolated from the colonial ascidian, *Synoicum turgens*. Turgencin $A_{Mox1}$ peptide with one oxidized methionine residue, inhibited the growth of melanoma cancer cell line. These natural peptides isolated from marine tunicates could have potential to act as promising drug leads [139].

Purified peptides from the ascidian, *Didemnum* has potential antibacterial effects against human pathogens [140]. Synthetic insulin dimers reduced blood glucose and swimming activity in zebrafish [141].

### 5.5. CONKUNITZIT

Novel conopeptides isolated from the venom of the cone snail, *Conus tilupia*. This peptide (ATLRNPSLCSLLPDTGSCRAAFHMFYFDQFSKECKVFIYGGCDGNANRFLNSKACYKTCGN) has an average mass of 9211.75. Conantokins and conopressins both failed to induce nirvana cabal effect. However, lower concentrations of this peptide antagonist reduced the escape response in zebra fish larvae [142].

### 5.6. ConoCAP

ConoCAP-vila was isolated from the venom of the cone snail, *Conus Villipinli*. ConoGAP-vila has a average mass of 1148.27. ConoCAP-a effectively decreased the heart frequency in drosophilia larvae and in rats. ConoCAP-a caused a decrease in blood pressure upon intraveneous administration in rat. Perfusion of rat ventricular cardiac myocytes with conoCAP-a caused a drop in the systolic calcium pressure. However, Cardiac negative inotropic effects were caused because of the impairment in intracellular calcium trafficking [143].

### 5.7. ConoGAY

ConoGAY-AusB was isolated from the venom of the cone snail, *Conus australis*. This peptide has an amino acid sequence GAYFDGFDVP**C**VPRRDD**C.** This peptide has an average mass of 2030.21**.** Lebbe et al., 2016 reported that, ConoGAY-AusB have no activity against the 29 Gram-negative, 10 Gram-positive bacterial strains and two yeast strains. AusB conopeptide consists of only a single disulfide bond, and was structurally different from that of other disulfide-poor peptides [144].

### 5.8. ConoNPY-Bt1

ConoNOY-AusB isolated from the venom of the cone snail, *Conus betulinus*. It has an average mass of 4391.05. ConoNPY conopeptide is an endocrine neurotransmitter found in the brain and also in autonomic nervous system of both the vertebrates and invertebrates. This peptide has capability of performing various processers such as blood pressure, energy balance, cognition, and epilepsy. However, several of these NPY receptors are G-protein coupled receptors (GPCRs). Biological activities were assayed upon intravenous injection of this peptide into mice brain. A dose of 20µg/mouse, showed signs of hyperactivity, such as tail flickering and jumping.

### 5.9. CONODIPINE

Conodipine –M alpha chain was isolated from the venom of the cone snail, *Conus magus*. This peptide catalyzes the calcium-dependent hydrolysis of the 2-acyl groups in 3-sn-phosphoglycerides. It has two chains, namely alpha and beta chains connected by disulfide bridges. Conodipine-M has an average molecular mass of about 13.6 kDa. Conodipine-M does not significantly discriminate phospholipids [145].

Conodipine M beta chain was isolated from the venom of the cone snail, *Conus magus*. It catalyzes the calcium dependent hydrolysis. Conodipine is composed of two chains, alpha and beta chains. Conodipine-M displayed phospholipase-A2 activity and it requires calcium as a cofactor. Conopeptide, Conohyal–Cn1 was isolated from *Conus consors*. Enzymatic digestion allowed the identification of conkunitzins (~ 7 kDa). This conopeptide was characterized and was found that it is similar to that of lactinoporin and hyaluronidase-like proteins [146].

### 5.11. CONOLYSIN

#### 5.11.1. CONOLYSIN- Mt1

Conolysins are conopeptides with an ability to disrupt the cell membrane intergrity. It was reported that, it was the first conopeptide with cytolytic activity. This peptide was isolated from *Conus mustelinus*. It is reported to be the new 23 amino acid conopeptide. It is a new 23 amino acid conopeptide. Conolysin-Mt showed hemolytic activity, when tested on human erythrocytes. Conolysin-Mt exhibited low antimicrobial activity (MIC > 50uM) towards two *Escherichia coli* strains [147].

### 5.12. CONOMAP- Vt

Conomap-Vt (Conp-Vt) was isolated from the venom of the cone snail, *Conus vitulinus*. This peptide displayed significant homology towards the peptides of the MATP (myoactive tetradecapeptide) family. Conp-Vt showed several potent excitatory activity[148]. Conomap-Vt (Conp-Vt) is a tetradecapeptide acting as endogenous neuromodulators in mollusks, annelids and in insects.

### 5.13. CONOMARPHIN –Eu1

This peptide was isolated from the venom of the cone snail, *Conus eburneus*. D-Phe13 group is needed for conomarphins activity and removal of post-translational modifications at Hyp 10 proline residue can be tolerated. It was reported, Conomarphin Eb1 administration in *P. Padulosa* caused sluggishness (>0.5 nmol), with minimal exposure of foot and cephalic tentacles (> 5 nmol), while elicited no effects on *Conus auratus* and mice [149].

Two variants of Conomarphin Eu1 have been found in the venom of *Conus eburneus*: GWVYHAHP(Gla)ONSFWT and GWVYHAHOEONSFWT [150].

## 6. CONCLUSION

Cone snails synthesize various biological venom components. These biological components are of significant interest to the pharmaceutical industries, to serve as analgesics and anti-inflammatories. Cone snail venom holds up thousands of different cono-peptides playing, a significant role in defending and prey capturing. Different families of these conopeptides possess specific properties towards the sodium channels and the nicotinic acetylcoline receptors, either blocking or activating them. Conopeptide synthesis takes place with the pre-pro peptide acting as the substrate, catalyzing the formation of mature peptide by proteolysis. These matured peptides have small structural units, which are known to modify ion channels. Most of these venom peptides have cysteine residues, capable of blocking ion channels. However, the properties of conopeptides, in terms of their flexibility and specificity towards ion channels, makes them an ideal souce for pharmaceutical industries as drug leads. All conopeptides mentioned have significant activity and specificity towards a variety of ion channels, inhibiting or increasing the efficiency of ion channels. Further, some of these conopeptides having good binding efficiency and being lipophilic have entered clinical trials. These host defense peptides, on the other hand serve as suitable drug molecules for curing Alzheimer, Parkinson and Epilepsy.

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