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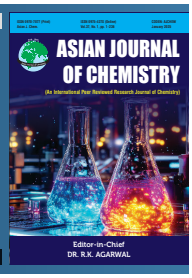

REVIEW

Amino Acids and Short Peptides Based Supramolecular Assembly

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Biological system uses supramolecular assembly for numerous important and complicated biochemical process in simple ways. One of the units of supramolecular assembly used by biological system is based on amino acids. Natural supramolecular assembly formed by amino acids are generally very long polymer of amino acids, which includes peptides, proteins, enzyme, *etc*. However, for amino acids-based synthetic supramolecular assembly, small number of amino acid units even in some cases single amino acid-based compound were used. It follows that synthetic nanomaterials based on amino acid-based supramolecular assembly can be designed to replicate the naturally occurring biological system and be used for a variety of purposes. Herein, due to their biocompatibility, biodegradability, simplicity of synthesis and rich chemistry of naturally occurring α-amino acids, short peptide-based supramolecular structures have been reviewed.

Keywords: Amino acid, Self-assembly, Peptide, Supramolecule, Gels.

INTRODUCTION

Enormous development has been done in past two decades in the field of interactions between the molecules *i.e.*, the field of supramolecular chemistry [1-6]. Scientists have prepared numerous supramolecular structures in the range of nanometer to micrometer scale using weak intermolecular forces like H-bonding, π–π interactions, hydrophobic interactions, electrostatic interactions and van der Waals forces *etc*. [7-10]. The morphology of the self-assembled structure can easily be tuned by changing the structure of the molecules, changing the chirality, altering the pH of the medium, changing the self-assembly condition, *etc*. [11-15]. Several morphologies of the supramolecular structures have been reported in the literature that includes nanotubes, nano sheet, ribbon, nanosphere, *etc*. [16-20]. These self-assembled structures have been utilized in several applications including electronic devices, biomedical devices, medicinal application, *etc*. [21-24].

Supramolecular assemblies and interactions play a crucial role in naturally occurring biological systems. Nature uses several functional materials that are produced by supramolecular interactions. The natural self-assembly includes DNA, peptide, protein, membrane of the different organism including cell membranes are prepared by self-assembly of lipid bilayers. Inspired by natural self-assembly, scientists have synthesized small molecules that follow the same principal of natural self-assembly to build superstructures which can be used in numerous applications. These small synthetic molecules mimic the natural molecules and use the weak intermolecular non-covalent interactions like π–π stacking, hydrophobic effect, hydrogen bonding, electrostatic interactions and van der Waals forces.

One of the most important building blocks to prepare supramolecular structures are the amino acid derivatives and short peptides because peptide molecules contain H-bonding donor amide (-CO-NH) and H-bonding acceptor carbonyl (C=O) groups. Thus, the probability of formation the supramolecular structure is much higher for the peptide molecules. Moreover, biodegradability, biocompatibility, ease of large-scale production makes peptide based supramolecular structure popular among the researchers. There are 20 naturally occurring α-amino acids. Peptides prepared from these amino acids show different properties depending on the side chain substitutions that include alkyl group, aromatic rings, hydrophilic groups, additional acid groups and additional basic groups. Thus, using these α-amino acids numerous numbers of short peptide molecules can be synthesized by changing the amino acids and sequences *e.g.* 8000

tripeptides, 160000 tetrapeptides and 3.2 million pentapeptides are possible from 20 naturally occurring amino acids. If we include unnatural amino acid like β-amino acid, amino acid with D-configuration, more diverse and complex supramolecular structures can be obtained. Since the side chains of amino acids may be tweaked to modify peptide properties, we have an abundance of potential peptides to choose from, allowing us to quickly adapt them to function demands. Thus, peptides are particularly suitable molecules that can be utilized to develop functional nanomaterials.

Previously peptides were synthesized only in solution phase which is hectic and time-consuming process. Also, by solution phase the yield of the peptide becomes very low specially for relatively long peptides. Solid phase peptides synthesis has been removed all the difficulties and now-a-days it becomes very easy and popular method to synthesis a reasonably long peptide with very short period of time with good yield [25,26]. Thus, using solid phase peptide synthesis large number of peptides have been synthesized and their self-assembled properties have been checked. Researchers also synthesized and checked the supramolecular properties of some more complex peptide molecules like cyclic peptides and dendrimer peptides [27-32]. Another group of researchers have developed the self-assembled peptide by system chemistry where they use the concept of out equilibrium synthesis [33-37]. Peptide bond formation and hydrolysis occurs in reversible pathway since the energy difference between peptide bond formation and hydrolysis is very small (~ 4 kJ mol^{-1}) [38]. A N-protected amino acid and a group of C-protected amino acid or *vice-versa* are mixed in an aqueous solution together with an enzyme, which can form/hydrolyze the peptide linkage. Very soon an equilibrium is reached by continuous interconversion between the building blocks and the reaction mixture is known as dynamic combinatorial library (DCL). Thus, after formation of amide bonds between the amino acids only that peptide in this DCL will prevail, which can form highly stable self-assembled structure and drive the equilibrium in the that direction *i.e.*, towards the formation of self-assembled peptides [36,37]. In this process we can synthesis the particular peptides which can give supramolecular structure in solution. By this process, a large number of self-assembled peptides have been synthesized. In some cases, the structure of self-assembled short peptides is inspired from a part of sequence of naturally occurring long peptides. It has been found that the part of naturally occurring peptide is responsible for the self-assembly of that peptide molecules *e.g.* the Phe-Phe (FF) sequence of Alzheimer causing β-amyloid is responsible for the fibril formation [39,40]. Inspired from this information the researchers have synthesized many short peptides having FF sequence and surprisingly most of the short peptide forms supramolecular structures in solution [41, 42].

In this review, the structures of short peptide and short peptide derivatives that can form supramolecular structure in different solvent will be discussed. In some cases, the self-assembled structures that leads to the hydrogelation of organogelation along with the spectroscopic and microscopic techniques that are generally used to characterize the self-assembly will also be discussed. Finally, the important applications of some of the supramolecular aggregates are also highlighted during the discussion of their self-assembly.

Self-assembly by single amino acid: Peptides have been used as one of the attractive scaffolds for synthesizing nanostructures. Peptide can give numerous nanostructures depending on the amino acid due to their varied chemical and functional diversity. Interaction between the amino acids residue in a peptide is so strong that there are few examples where even a single amino acid can form supramolecular structure under suitable condition [43-46]. The pioneering work reported by Abramovich *et al.* [43] that simple L and D-phenyl alanine amino acid (**1** and **2**, Fig. 1) can aggregate to form fibrillar structure in solution. They confirmed the presence of fibrillar structure by transmission electron microscope (TEM), scanning electron microscope (SEM) and also confocal microscopy image using the dyes thioflavin T and Congo red. Later on, Perween *et al.* [44] reported that phenylalanine as well as L and D-tyrosine (**3** and **4**) can also form long, fibre-like aggregates at very low concentration such as 1 mM in deionized water. The aspect ratio of the fibres were found to be very high as the widths are ranging from 300-800 nm and the length was on the order of several micrometers. They also reported that no fibres were observed at very low concentration like 1 μM. The chirality of the supramolecular structure was confirmed by circular dichroism (CD). It was found that both L-phenylalanine (**1**) and D-phenylalanine (**2**) shows CD signal at 218 nm but in opposite direction, which proves the inverted supramolecular chirality in the super structure. Actually, this supramolecular chirality in transmitted from the molecular chirality of the amino acids. They also reported that glycine (**5**) does not show any fibrillar structures and also not respond to circular dichroism experiment. Gour *et al.* [45] reported for the first time that non-aromatic amino acid cysteine and methionine can from amyloid like self-assembly in aqueous solution. By SEM images they confirmed fibrillar network of the assemblies with fiber length few micrometers and width ~200 nm.

Self-assembly by single amino acid derivatives: Due to their relative ease of synthesis, supramolecular structures based

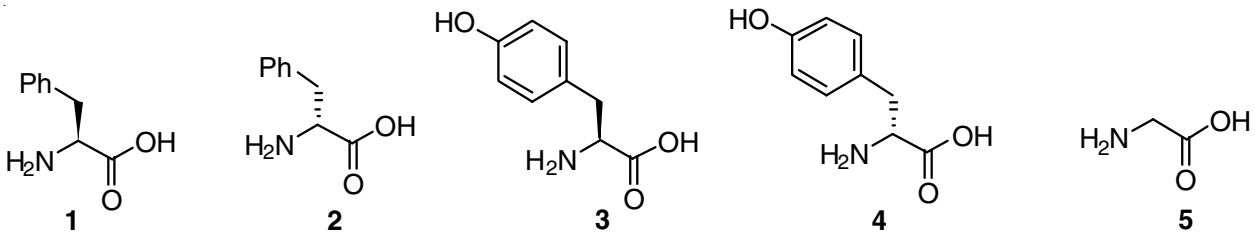

Fig. 1. Chemical structures of amino acids, **1**-**5**

on single amino acids have recently attracted a lot of attention for development. Derivative of single amino acid should not be considered as peptides since these molecules do not have any -CONH- bond between two amino acids. There are many types of simple single amino acid based supramolecular structure are reported in the literature. The derivatives of the amino acids are prepared by various ways such as (i) attachment of long alkyl chain at the N-terminal of the amino acid (ii) attachment of aromatic moiety at the N-terminal of the amino acid, and (iii) attachment of long alkyl chain at C-terminal and making ionic at N-terminal.

(i) Attachment of long alkyl chain at the N-terminal: Preparation of supramolecular structure by protection of N-terminal end with a long chain has also been reported in the literature. By this modification, the amino acid is converted to an amphiphilic molecule where the long chain acts as hydrophobic tail and amino acid acts as polar head. One of the early reports, Bhattacharya & Krishnan-Ghosh [47] attached *n*-lauric acid (C-12 chain) to the N-terminal of L-alanine to form N-lauroyl-L-ala-nine (**6**, Fig. 2). This molecule forms supramolecular entangled network in many organic solvents like petrol, kerosene, benzene, toluene, xylenes, cyclohexane, *etc*. SEM images of **6** in heptane and toluene confirms the formation of fibres with different thickness. The authors confirmed that the presence of H of -COOH and -NH- groups are essential for the formation of supramolecular structures. To understand the mechanism of self-assembly, they synthesized two more compounds (**7** and **8**), which do not show any supramolecular properties. Thus, the H-bonding between the amino acid moieties is prerequisite for the formation of supramolecular structure for these types of molecules.

Pal and Dey [48] have also reported the formation of supramolecular structure by amino acid derivative, where they modified the amino acid into a urea like structure (**9**-**11**, Fig. 2). The strong driving factor of supramolecular structure formation by this urea like derivative has two H-bonding donor -NH- and one H-bonding acceptor carbonyl (C=O) group. They have designed and synthesized a series of urea like derivative (**9**, **10**, **11**) using L-alanine amino acid with different chain length and checked their ability to form supramolecular structure in solution phase. They observed that these compounds form supramolecular structure only when dissolved under heating in chlorinated solvent or aromatic solvent in presence of small amount of water, methanol or urea. The authors concluded that these amphiphilic molecules form intermolecular H-bonds through the water molecules in between them (Fig. 3). Gao *et al.* [49] also reported that N-stearoyl-L-glutamic acid (C_{18}-Glu) (**12**) forms nanostructure having disk and fibre

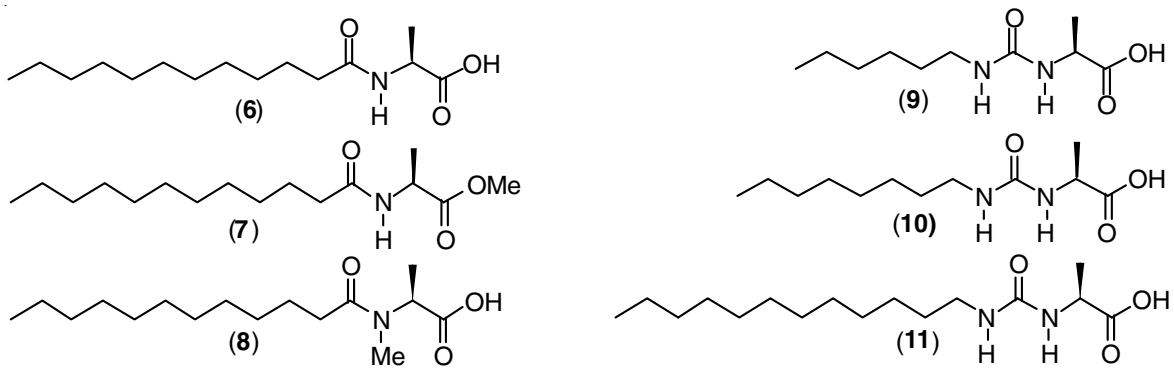

Fig. 2. Chemical structures of N-terminal long alkyl chain substituted single amino acid derivative **6**-**11**

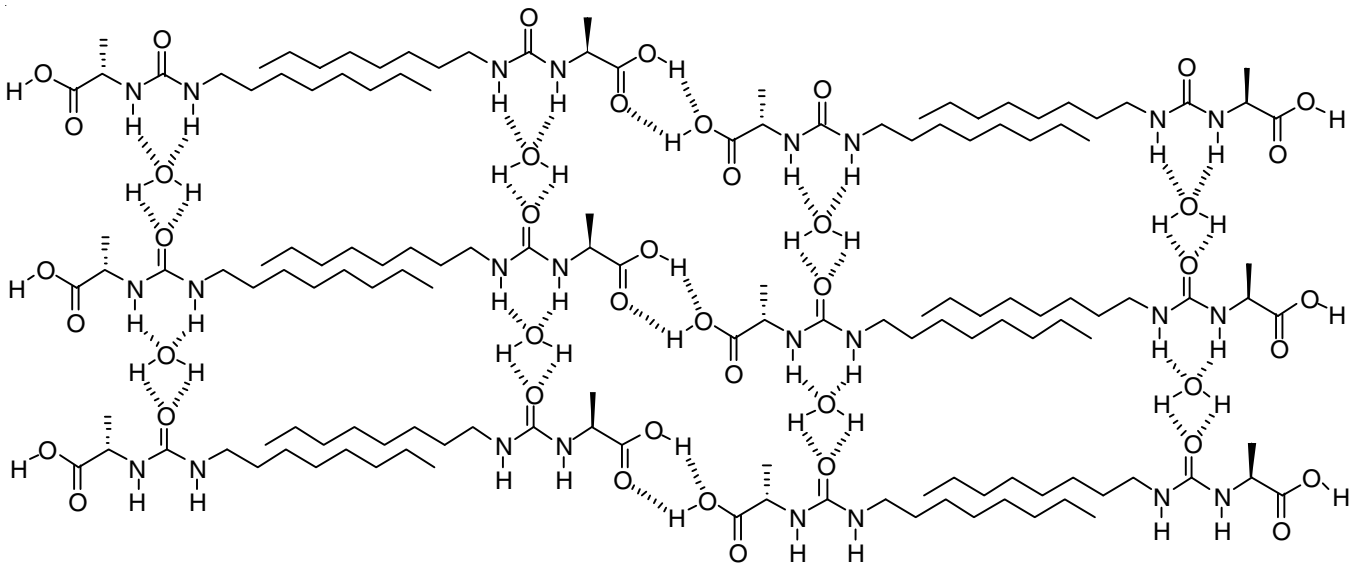

Fig. 3. Schematic representation of H-bonding of N-terminal substituted single amino acid derivative

morphology in both hydrophobic and hydrophilic solvent through the intermolecular and intramolecular H-bondings.

(ii) Attachment of aromatic moiety at the N-terminal of amino acid: Attaching an aromatic moiety on the N-terminal end of the amino acid can produce very strong π–π interaction to stack the aromatic groups in a particular arrangement, which actually creates much stronger attractive interaction than that of between long alkyl chain. Nature also uses this π–π stacking interactions within the polypeptide chain to attain a particular ordered structure of the proteins and enzymes [50]. Inspired from these natural examples, researchers also have developed some synthetic system where amino acid is ligated to an aromatic moiety in the N-terminal end to develop beautiful supramolecular structures that has several electronics and biomedical applications. There are number of examples in literature where a single amino acid is attached to a large aromatic moiety to produce supramolecular structure in various solvents [51-55]. The mostly used aromatic moieties are fluorenyl, ferrocenyl, naphthyl and pyrenyl derivatives. The earliest report published by Xu & Yang [51] who showed that fluorenylmethyloxycarbonyl (Fmoc) conjugated with tyrosine (**12**, Fig. 4) can form strong supramolecular aggregates in water in alkaline pH. The supramolecular aggregation was visualized by scanning electron micrograph, which showed uniform fibres with width 200-600 nm. Later, Nanda *et al.* [52] synthesized a pyrenyl derivative of phenylalanine (**13**, Fig. 4), which gives very strong supramolecular aggregates that visually appear as thixotropic hydrogel. Strong π–π interaction between the pyrene ring as well as between the phenyl rings helps for the formation of this supramolecular structure. The importance of the phenyl ring of phenylalanine were understood from the fact that they also synthesized another pyrenyl derivative with valine amino acid (**14**, Fig. 4), which does not show any supramolecular structure. Sun *et al.* [53] serendipitously discovered that ferrocenoyl phenylalanine (**15**, Fig. 4) aggregates in water to form very stable superstructure that ultimately form robust hydrogel which responses toward multiple stimuli like redox potential and pH of the solution. Using DFT calculation, they concluded that the ferrocenoyl group of one molecule interact with phenyl ring of second molecule *via* π–π stacking interaction and the -COOH groups form intermolecular H-bonding to give an antiparallel, non-covalent brick like dimer. These millions of dimer aggregates with one another to form the fibrillar network which restrict the movement of water causing the hydrogelation. Since the molecule contains a ferrocene group, it shows sharp reversible sol-gel phase transition towards the change of redox potential of the solution. Due to presence of -COOH group, a sharp sol-gel transition is also found with the change of pH. Recently, Bassan *et al.* [54] reported the attachment of simple naphthalene unit at the N-terminal of phenylalanine can give strong supramolecular aggregate due to strong π–π interaction between the naphthalene unit as well as intermolecular hydrogen

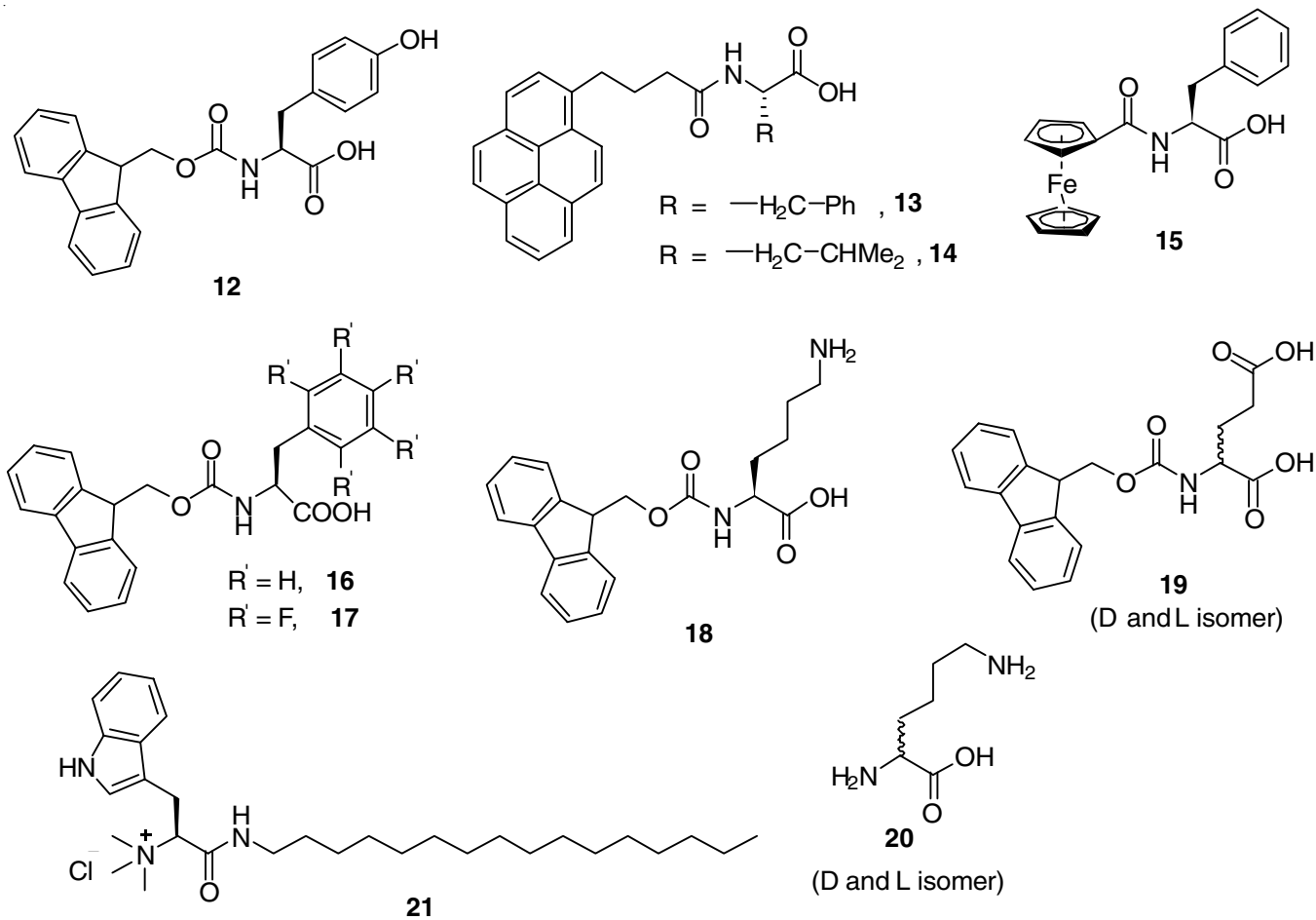

Fig. 4. Chemical structures of N-terminal aromatic group substituted single amino acid derivative **12**-**21**

bonding between the -COOH groups. Ryan *et al.* [55] reported that attachment of aromatic group with modified amino acid also led to very strong self-assembly that form strong hydrogel. This group synthesized Fmoc-pentafluorophenylalanine where the five H of the phenyl ring of phenylalanine is replaced by five F atoms (**16-17**, Fig. 4). It is found that compound **17** forms supramolecular hydrogel at very low concentration (0.1 wt.%) within few minutes whereas its unsubstituted counterpart, Fmoc-phenylalanine (**16**) fails to give supramolecular structure under similar condition. It is hypothesized that the unique hydrophobic and electronic nature of fluorene helps for strong self-association in solution.

There are also some reports in literature where mixture of Fmoc-amino acids forms very strong self-assembly. Xu *et al.* [56] reported that mixing of 1 equiv. of Fmoc-Lysine (**18**, Fig. 4) with 1 equiv. of Fmoc-phenylalanine (**16**, Fig. 4) in presence of 2 equiv. of Na_2CO_3 in water gives very strong self-assembled structure. Banerjee *et al.* [57] also showed that the two oppositely charged Fmoc protected glutamic acid (**19**) and lysine (**20**) form stable supramolecular aggregates in water. The chirality of the amino acids was also changed. It has been found that when both the amino acids are L-isomer, the fibres become left-handed whereas when both the amino acids are D-isomer the fibre become right-handed. This handedness was confirmed by circular dichroism spectroscopy, which showed that the spectra given by D-amino acids mixture (D-isomer of **19** and **20**) is exactly mirror image with the spectra obtained from the mixture of L-amino acids (L-isomer of **19** and **20**). Left-handed and right-handed helical fibers were also clearly visualized by AFM images. It has been observed that a mixture of Fmoc-racemic-glutamic acid and racemic-Lysine also forms a supramolecular structure, with self-sorting occurring between the D-isomer and L-isomer. Therefore, both left-handed and right-handed helical fibers were seen in nearly equal quantities, and the circular dichroism experiment yielded no signals.

(iii) Attachment of long alkyl chain at C-terminal and making ionic at N-terminal: There are some reports in the literature where supramolecular structures with amino acids are formed by the protection of C-terminal by a long chain and converted the N-terminal into an ammonium salt. Das *et al.* [58] for the first time reported that the conversion of acid group of tryptophan amino acid to an amide with cetyl amine ($C_{16}H_{33}$-NH_2) and the amine group to trimethylammonium chloride gives an amphiphilic molecule (**21**), which can give strong supramolecular structure in water at very low concentration. The supramolecular fiber formation was confirmed by scanning electron microscopy and circular dichroism spectroscopy. It has been found that the aggregation is formed by π–π stacking between the indole moieties and intermolecular H-bonding between the amide-H and amide carbonyl. It has been confirmed that the amide-H and indole-NH are highly essential for the formation of supramolecular structure since the molecules without the amide-H and indole-NH do not give self-assembly.

Self-assembly by dipeptide: There are few examples in literature where it has been reported that some specific dipeptide can self-assembled in solution to give excellent robust supramolecular structure [59,60]. Reches & Gazit [59] for the first time reported that diphenylalanine dipeptide (Fig. 5) can give robust hollow nanotube in water. These nanotubes are discrete, highly rigid, shows very high aspect ratio. The major driving force for the discovery of the nanotube is that the scientists observed the main structural motif for Alzheimer causing β-amyloid fibril formation is the Phe-Phe sequence in the polypeptide chain. Inspired by this observation they synthesized only Phe-Phe dipeptide and found that this dipeptide also forms nanotubular structures in solution. These nanotubes can easily be formed by dilution the organic solution of Phe-Phe dipeptide with water or heating of its water solution followed by cooling down. The hollow nanotube has been used as scaffold for the fabrication of silver nanowires that is potentially useful for the electronic devices. Silver nitrate solution was added to the nanotube solution. Being hollow, the Ag^+ ions are accumulated inside the nanotube. Then the Ag^+ ions are reduced with citric acid to form silver nanowires inside the peptide nanotube. The peptide nanotube filled with Ag nanowires were then incubated with proteinase K enzyme to destroy the peptide nanotube by hydrolysing the peptide bond. TEM images of the solution after incubation confirmed the presence of silver nanowire with uniform width and length. These silver nanowires confirmed the formation of uniform hollow nanotube by the Phe-Phe dipeptide.

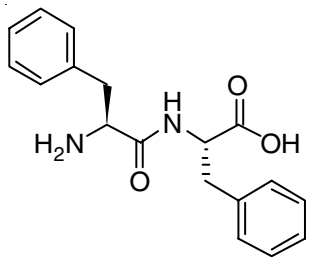

Fig. 5. Chemical structure of pure dipeptide Phe-Phe

Self-assembly by dipeptide derivatives: Lots of dipeptide derivatives are reported in literature, which can form stable self-assembled structure in solution. Following are the major three types of dipeptides based molecules which show self-assembly properties; (a) an aromatic group or long alkyl chain is capped at the N-terminal of the dipeptide, (b) an aromatic group or long alkyl chain is capped at the C-terminal of the dipeptide and converting amine group to ammonium salt, (c) an aromatic group or long alkyl chain is capped at the N-terminal of the dipeptide and converting the C-terminal as ionic group; and (d) both the N-terminal and C-terminal of the dipeptide are attached with long alkyl chain.

(a) N-terminal capped dipeptides: The most common dipeptide derivation to induce self-assembly in solution is the capping of N-terminal with some large aromatic group like Fmoc, naphthalene, anthracene, *etc*. [61,62]. Xu *et al.* [61] for the first time reported that Fmoc-D-Ala-D-Ala (**22**, Fig. 6) and Fmoc-L-Ala-L-Ala (**23**) forms strong supramolecular aggregate in water at pH 3 at very low concentration. The supramolecular aggregates are so strong that the solution ultimately forms self-supportive hydrogel. Some other Fmoc based dipeptide like compounds **24**, **25** and **26** also form hydrogel at the relatively higher concentration.

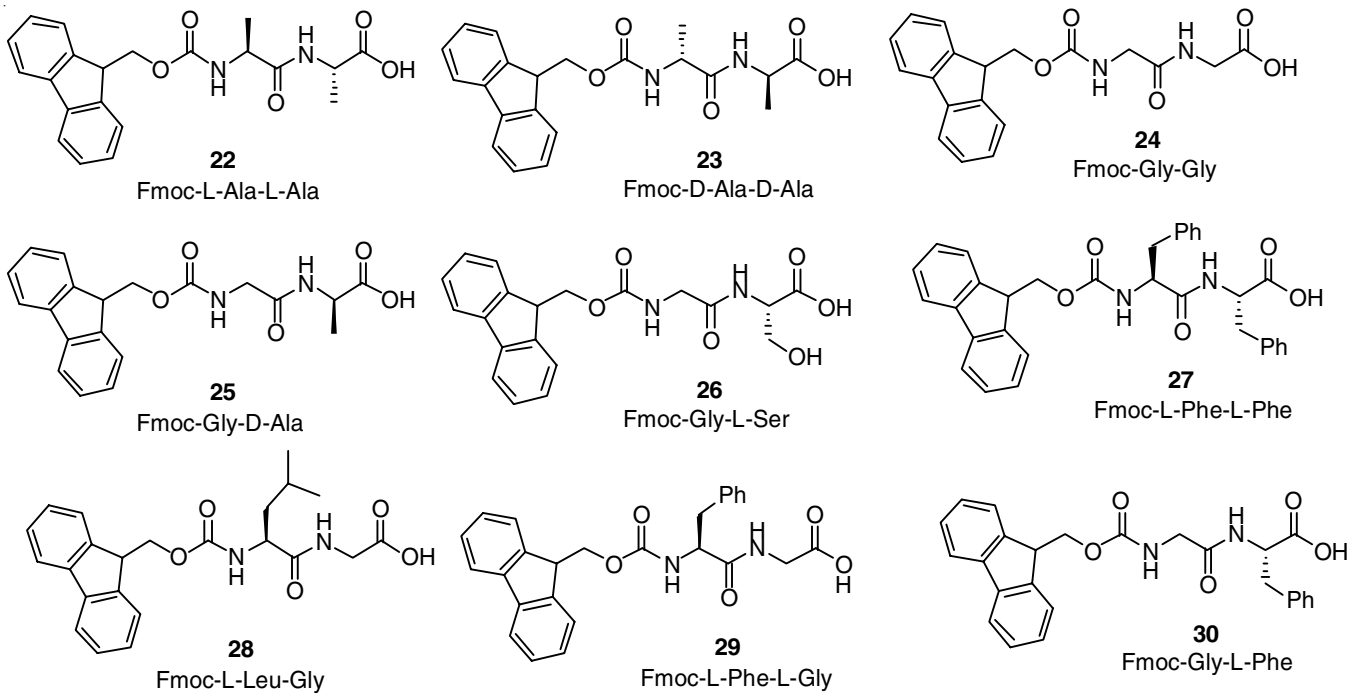

Fig. 6. Chemical structures of N-terminal Fmoc substituted dipeptides **22**-**30**

Ulijn *et al.* [63,64] reported some other Fmoc-dipeptide molecules, which forms supramolecular structure at pH lower than 4 at 20 mM concentration. They reported that compounds **28** and **29** form supramolecular structures if the water dispersion of these compounds are dissolved by adding conc. NaOH followed by the pH is lowered by adding HCl to this solution [63]. Compounds **27**, **28** and **29** form clear self-supporting hydrogel at this pH. The anti-parallel β-sheet type of arrangement between the dipeptide molecules were confirmed by the peak at 218 nm in the circular dichroism spectra for Fmoc-Phe-Phe (**27**) molecule [64]. They also reported another peak at ~305 nm for the π–π^* transition of the fluorenyl group. Interestingly, Fmoc-dipeptides of achiral amino acid (**24**) does not show any CD signal.

Another series of dipeptide based supramolecular structure has been reported where the dipeptide is conjugated with naphthalene moiety (**31-36**, Fig. 7). Large number of dipeptides having different amino acid sequence has been used [65]. It has been found that few glycine-based dipeptides like **31**, **32**, **33** and **34** give strong supramolecular structure to form self-supportive hydrogels at very low concentration. The other dipeptides in this series do not form any supramolecular structure. The supramolecular chirality of the superstructure has been confirmed by circular dichroism (CD). It has been found that the peptide with D-amino acid (**32**) and the peptide with L-amino acid (**33**) give left-handed and right-handed self-assembled nanofibers respectively in the supramolecular structures.

(b) C-terminal capped dipeptides: Das *et al.* [66] reported the formation of supramolecular structure by dipeptides where the C-terminal of the dipeptide is capped with a long alkyl chain and the N-terminal side is converted to more water-soluble ammonium salt. Interestingly, it was found that almost all the dipeptide of this series forms strong supramolecular network in water. Molecules **37-40** (Fig. 8) form transparent hydrogel. Several microscopic techniques like SEM, TEM, AFM were used to find the supramolecular network. These dipeptide-based hydrogels later used for antimicrobial activity against gram positive as well as gram negative bacteria [67].

(c) N-terminal capped and C-terminal ionic dipeptides: There are several reports in literature where N-terminal of the dipeptide is attached with a long alkyl chain or large aromatic groups and C-terminal of the dipeptide is converted to anionic

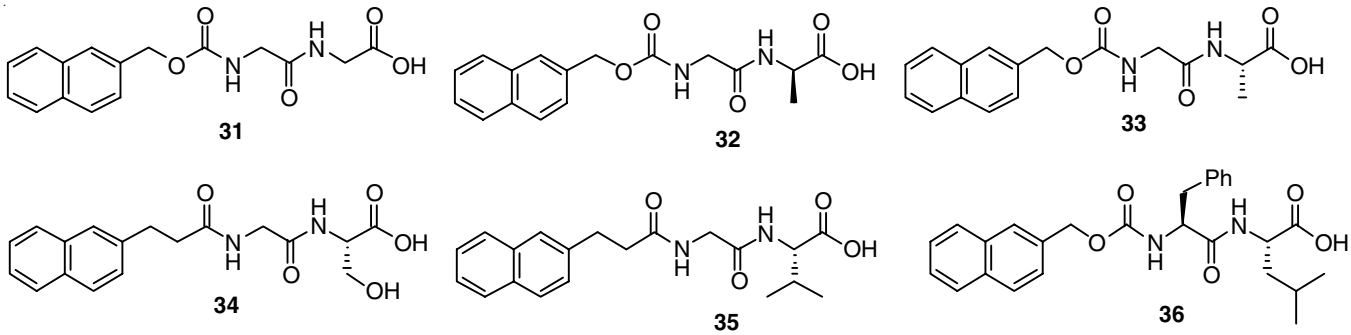

Fig. 7. Chemical structures of N-terminal Naphthalene substituted dipeptides **31**-**36**

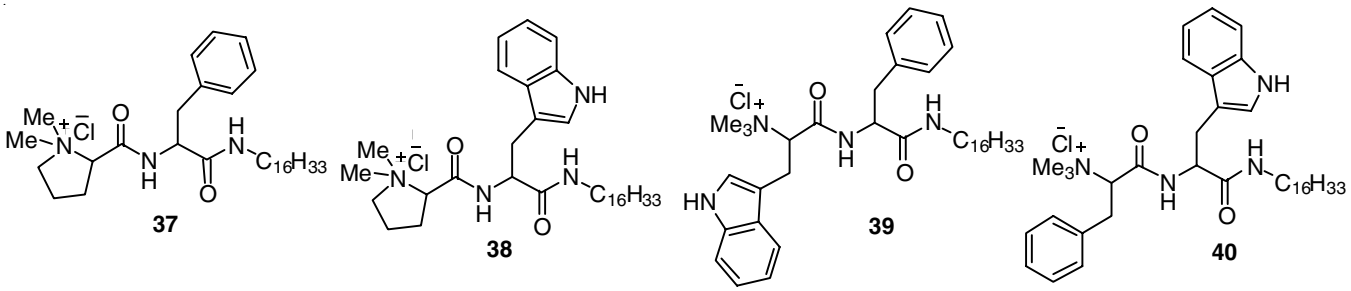

Fig. 8. Chemical structures of C-terminal long chain substituted quaternary ammonium dipeptides **37**-**40**

or cationic systems [68,69]. Das *et al.* [68] reported a series of dipeptide (**41**-**44**, Fig. 9) which is protected in the N-terminal side by coupling alkyl chain and the C-terminal end is converted to corresponding carboxylate by addition of equivalent amount of NaOH. Therefore, by these reactions these molecules are converted to an amphiphilic dipeptide with an anionic head-group. These molecules were found to form supramolecular structures in water as well as in various organic solvents like toluene, tetralene, xylene, carbon tetrachloride, *etc*. Later-on, the same group [69] reported another series of dipeptide-based molecules where the N-terminal of the peptide is capped with large aromatic Fmoc group and the C-terminal is converted to pyridinium salt (**45**-**48**). By this process, the dipeptide is converted to a cationic amphiphilic dipeptide. Interesting, these compounds were also found to form supramolecular structure in water at very low concentration. Antiparallel β-sheet arrangement in the supramolecular structure were confirmed by several spectroscopic and microscopic studies. This series of dipeptide molecules were further used as antibacterial agent against both Gram-positive and Gram-negative bacteria.

(d) Both N-terminal and C-terminal of dipeptide attached with long alkyl chain: Das *et al.* [70] also reported another series of dipeptides, which were protected on both C-terminal and N-terminal with long carbon chain (**49**-**54**, Fig. 10). It has been found that all these dipeptides derivative form very strong supramolecular structure in organic solvents like

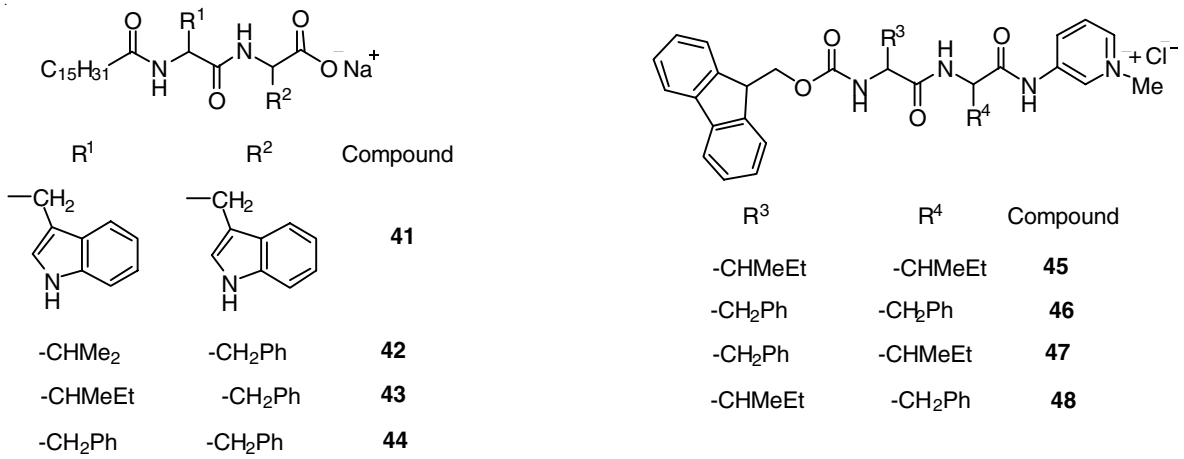

Fig. 9. Chemical structures of N-terminal substituted ionic dipeptides **41**-**48**

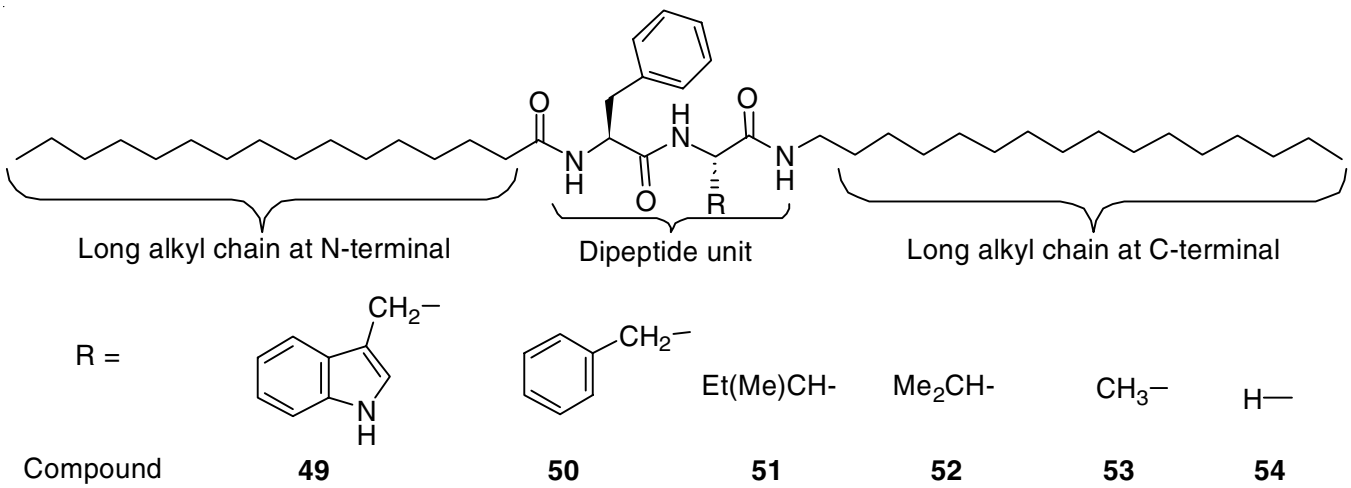

Fig. 10. Chemical structures of both N and C-terminal long chain substituted dipeptides **49**-**54**

toluene, tetralene, xylene, chlorobenzene, *etc*. It has also been reported that the efficiency of supramolecular structure formation is highly dependent on the dipeptide unit and the length of the alkyl chains on both C-terminal and N-terminal. The results showed that the Phe-Gly dipeptide (**54**) is the most efficient unit for self-assembly. Also, efficiency for the supramolecular structure formation increases with increase of the chain length of two terminals. Later on, the xerogel of the supramolecular structure were utilized for the purification of water. The xerogels were used for removal of positively charged dye crystal violet and negatively charged dye rhodamine 6G from water. It has been concluded that the dye molecules are absorbed between the fibres of the supramolecules which was confirmed form SEM images of the xerogel after dye absorption.

Self-assembly by tripeptide and tripeptide derivatives: There are some reports in literature where uncapped tripeptide can form supramolecular structure in water [71-75]. Tamamis *et al.* [72] for the first time reported that a tripeptide FFF (**55**, Fig. 11) (Phe-Phe-Phe) forms planar nanostructure with β-sheet arrangement when the compound is dissolved in fluorinated alcohol and then diluted in water. The β-sheet arrangement were confirmed by various spectroscopic and microscopic techniques. It is well known that amyloid peptide shows self-assembly to form fibrils. Inspired from the self-assembling behaviour of amyloid fibrils, scientists find out the specific sequence of amino acid, which is responsible for the fibre formation. One of the sequences of amyloid fibrils formation is KLVFF (K = lysine, L = leucine, V = valine, F = Phenyl alanine). The self-assembling behaviour of FF (Phe-Phe) dipeptide is already discussed in the dipeptide section. Marchesan *et al.* [73] showed that the uncapped tripeptide from amyloid peptide sequence can also give very strong supramolecular structure in water. For that purposes, this group has used Val(D)-Phe-Phe (**56**) sequence of tripeptide which undergoes very strong self-assembly in water that visually appears as self-supportive hydrogel at physiological pH. They also synthesized another tripeptide Phe(D)-Phe-Val (**57**) which also showed self-supportive gelation in water. CryoTEM of the tripeptides confirmed the presence of nanoscale morphology of fibres. Interestingly, tripeptide **56** gave long parallel nanotapes whereas tripeptide **57** self-assembled into twisted fibers that appears as thicker filaments. Singh *et al.* [74] reported that when a tyrosine residue is attached to FF dipeptide to form two tripeptide YFF (**58**) (Phe-Phe-Tyr) and FFY (**59**) (Tyr-Phe-Phe), both the tripeptide showed self-assembling behaviour in ethanol. These two molecules can be considered as the derivative of FFF (Phe-Phe-Phe) tripeptide since compounds **58** and **59** are obtained by addition of a OH group at the *para*-position of phenyl ring of N-terminal and C-terminal phenylalanine, respectively. This minute change in the structure brings huge change in supramolecular behaviour. Histidine containing tripeptide **60** that forms supramolecular structure has been reported by Garcia *et al.* [75]. The peptide conformation in solution was confirmed to be β-sheet by FTIR and CD. Interestingly, the supramolecular aggregate was utilized as biocatalyst for the hydrolysis of ester. The histidine unit is the most essential amino acid in the active center of the biocatalyst since it plays a role of proton donor or acceptor in ester hydrolysis.

Some protected tripeptides have also been reported in literature, which can give strong supramolecular interactions in

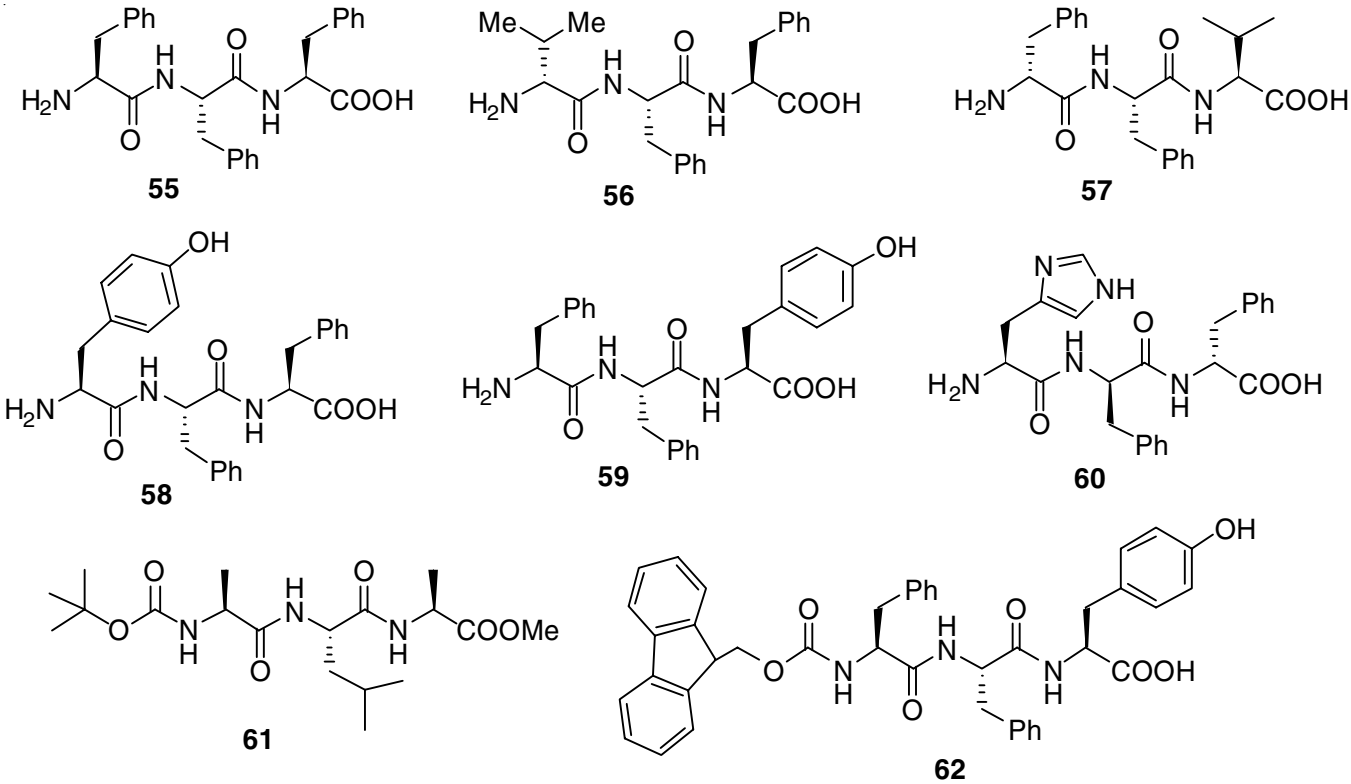

Fig. 11. Chemical structures of tripeptides and tripeptides derivatives **55**-**62**

solution. Das *et al.* [76] reported that Boc-Ala-Leu-Ala-OMe (**61**, Fig. 11) forms antiparallel hydrogen-bonded dimers which self-associate to form supramolecular β-sheet structures in crystals and amyloid-like fibrils in the solid state. Scanning electron micrograph showed the amyloid like fibrillar morphology in the solid state. The authors also confirmed from crystal structure that the intermolecular H-bonding between self-assembled dimer forms the 2D monolayer antiparallel β-sheet structures. Criado-Gonzalez *et al.* [77] reported that Fmoc-FFY (**62**, Fig. 11) forms self-assembled supramolecular hydrogel in water. The compound forms β-sheet type of structure *via* self-assembly through π–π stacking interactions, hydrogen bonds and hydrophobic interactions. Cryo-SEM showed that the molecules undergo self-assembly to form fibrillar network with a thickness of few micrometers. Later, it has been shown that the self-assembles network can fully inhibit to the development of Gram-positive *Staphylococcus aureus* bacteria.

Self-assembly by tetrapeptide and tetrapeptide derivatives: Some tetrapeptides and tetrapeptide derivatives which self-assembled into supramolecular structure are reported in literature. Sivagnanam *et al.* [78] reported that a tetrapeptide and its Lys-Val-Ala-Val (**63**, Fig. 12) and its Boc-protected analogue (**64**) can form self-assembled supramolecule to form spherical structure in 1:1 aqueous ethanol solvent system. These spherical aggregates are found to encapsulate the small drug molecule like doxorubicin and interestingly it was observed that the doxorubicin loaded spherical aggregates can penetrate the cell membrane to show intracellular drug delivery capacity. Liang [79] *et al.* reported that coarse-grained (CG) molecular dynamics predicted that Alzheimer's β amyloid peptide (Aβ peptide) VFFA (**65**) (Val-Phe-Phe-Ala) could self-assemble into nanosphere and its derivative FFFA (**66**) (Phe-Phe-Phe-Ala) could self-assemble into nanosheet. Kurbasic *et al.* [80] reported two tetrapeptide molecules (**67**, **68**) involving first and second amino acids from N-terminal are D isomer and third and fourth amino acids are L-isomer [80]. These two molecules show strong self-assembly in water at neutral pH. The tetrapeptide contains Ser-His motif for catalytic activity and Phe-Phe motif for promotion of amyloid self-assembly. The self-assembling structure has been characterized by CD, TEM and rheology. The self-assembled peptide **67** shows strong CD signals around 255-275 nm and in TEM both the peptide show fibrous structure having width 470 and 280 nm for peptide **67** and **68** (Fig. 12), respectively. Interestingly, the peptide can mimic hydrolase enzyme to show catalytic activity towards hydrolysis of 4-nitrophenyl acetate to release *p*-nitrophenol. The catalytic activity may come from the serine and histidine residue of the peptide, which is present in most of the natural enzyme in their active site.

Some derivatives of tetrapeptides are also reported in the literature, which show self-assembled supramolecular structure in various solvents. Castelletto *et al.* [81] showed that two tetrapeptides (**69**, **70**, Fig. 13) appended with Fmoc group at N-terminal can form very strong self-supportive hydrogel in water. From TEM images, it has been shown that the molecules form very thin fibrillar structures with average width only 10 nm. The self-assembled structure in the peptide molecules is dominated by π–π interaction between the Fmoc units and H-bonding between the peptide chains were confirmed by CD and FTIR spectroscopy. Cui *et al.* [82] reported four tetrapeptide molecules attached with a long chain in the N-terminal of the peptide (**71**, **72**, **73**, **74**, Fig. 13). The four tetrapeptides are formed by using two amino acids (V = valine and E = aspartic acid) with different sequence in the peptides. The peptide solutions are prepared in weakly alkaline medium (1 mM NaOH) followed by aging for 2 weeks at room temperature in order to eliminate possible kinetic effects on the self-assembled nanostructures. Interestingly, it has been found that the supramolecular structures of the peptides were also changed significantly with the change of peptide sequence. In cryo-TEM, it has been found that peptide **71** with VEVE sequence the self-assembled structure gets nanobelt morphology with average width 120 nm whereas peptide **72** with EVEV sequence forms twisted ribbon with average width 60 nm. The most significant change in morphology occurs when the position of middle two amino acids are interchanged. Peptide **73** with VVEE and peptide **74** with EEVV sequence forms nanofibers with average width 9 and 18 nm, respectively.

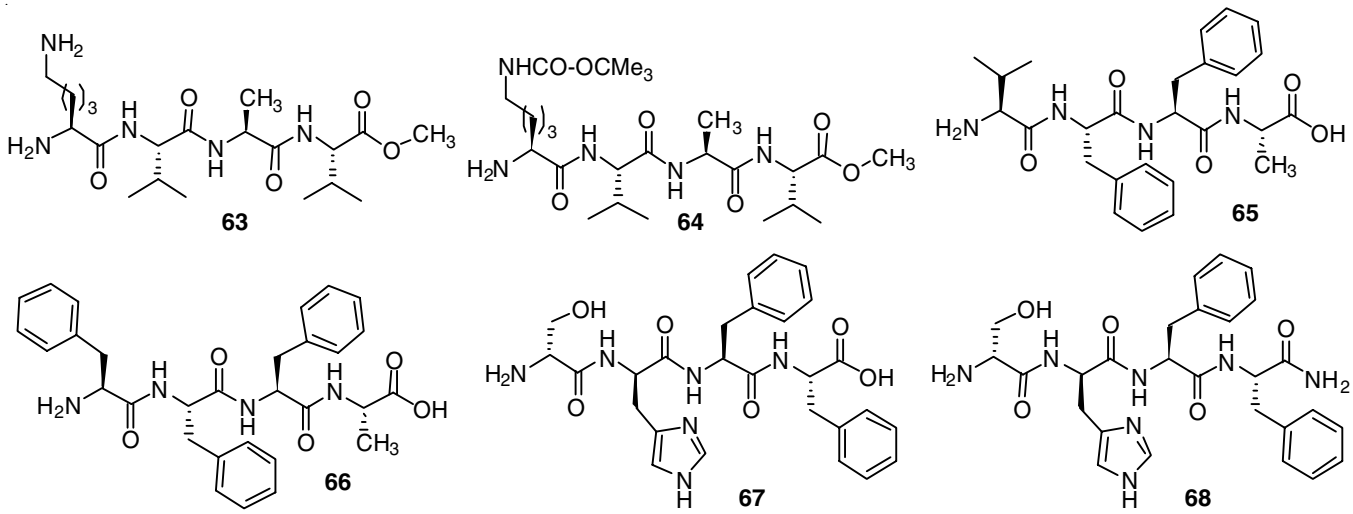

Fig. 12. Chemical structures of tetrapeptides **63**-**68**

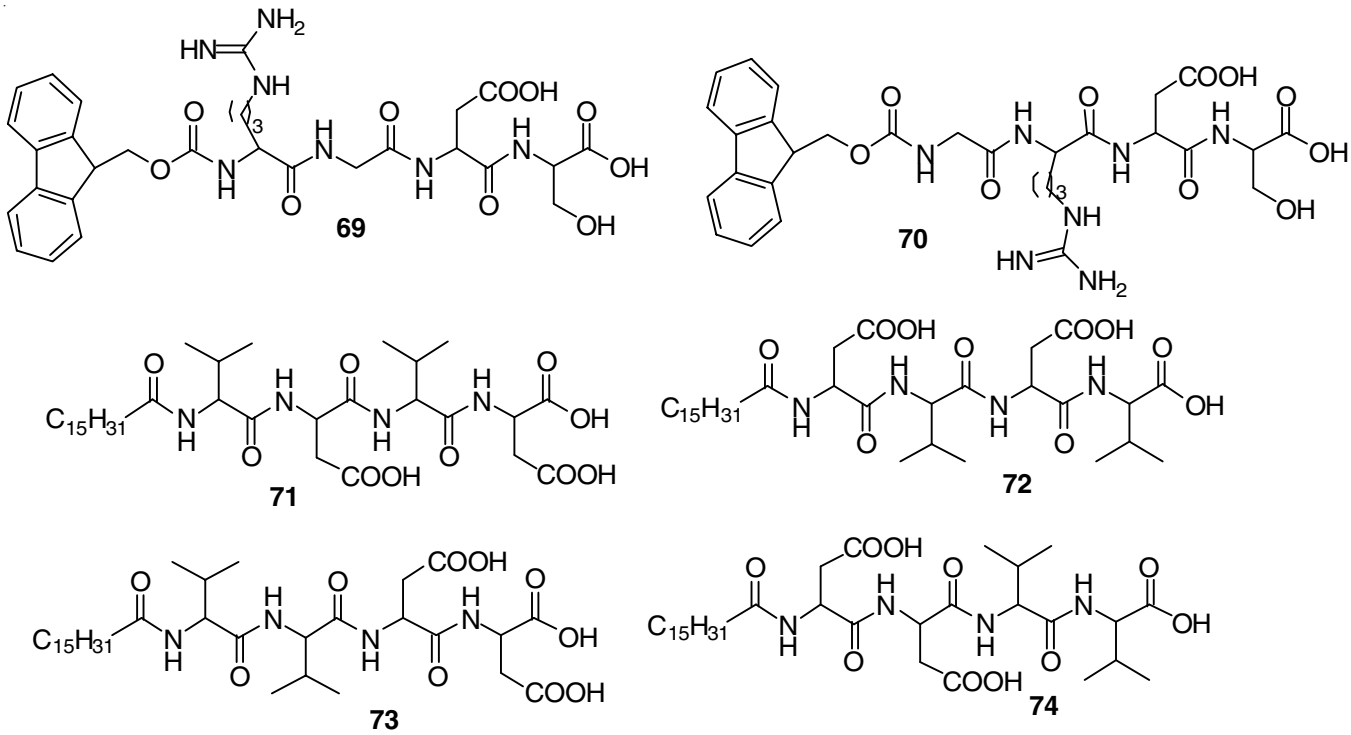

Fig. 13. Chemical structures of terrapeptides derivatives **69**-**74**

Self-assembly by pentapeptide and pentapeptide derivatives: Few pentapeptides have been reported in literature which forms supramolecular structures in different solvents. One of the initial reports came from Tan *et al.* [83]. They have reported a pentapeptide (**75**, Fig. 14), which was capable of forming supramolecular structure in ethanol solvent. The TEM and AFM images confirmed the entangled fibrillar network between the peptide moiety in the self-assembled state. It was observed that the change of solvent from water to ethanol induces red shift from 274 to 277 nm in UV-Vis spectroscopy, which confirm the π–π stacking between the aromatic groups in the self-assembled state in ethanol compare to non-self-assembled state in water. The similar aggregation induced enhancement of emission is also observed in ethanol confirming the π–π stacking between the aromatic groups. Tang *et al.* [84] reported a series of pentapeptides that form nano fibrillar structures water leading to hydrogelation. Interestingly, the peptide sequence, pH and peptide concentration play an important role for the mechanical properties of the gels. After using different sequence, it has been observed that phenylalanine and leucine are the most important amino acids that need to be present in the pentapeptide sequence for inducing gelation at pH 7.4 PBS buffer. Two most important pentapeptides sequence that form robust hydrogel under this condition are AYFIL-NH_2 (**76**) and KYFAL-NH_2 (**77**, Fig. 14). Jitaru *et al.* [85] reported another pentapeptide derivative FEYNF-NH_2 (**78**), which forms hydrogel under physiological condition. Clarke *et al.* [86] reported that a pentapeptide (**79**, Fig. 14) conjugated with arylated methyl-viologen gives β-sheet self-assembly which ultimately form conductive nanofibers. Using several spectroscopic and microscopic techniques, they proved that the aryl-viologen part of the molecules remain in close contact to form a less twisted parallel β-sheet. Atomic force microscopy confirmed the semiconductive nature of the nanofibers compare to amorphous aggregate of the same molecules. The authors proposed that the conductivity of the self-assembled nanofibers comes from reduction of the viologen portion of the molecules followed by transport of electrons between the viologen part along the supramolecular stack.

Conclusion

Short peptide based supramolecular structures have gain significant interest among the researchers, since peptide has innate properties of biocompatibility, biodegradability, ease of synthesis and very rich chemistry of 20 naturally occurring α-amino acids. Short peptides can self-assembled into various superstructures including nanotube, fibres, ribbons tapes, *etc*. These superstructures can be utilized in various electronic and biomedical applications. Thus, to develop a peptide based nanomaterials, the relation between structure of the molecules with their possibility of the formation of the superstructures should be elucidated. A lot of short peptides based supramolecular structures have been discussed with the variation of both N-terminal and C-terminals. Dipeptides and tripeptides molecules are very likely to form superstructures if there is a large aromatic substitution like Fmoc, naphthyl, pyrenyl, *etc*. in the N-terminal end. Similarly, long alkyl chain on N- or C-terminal makes the molecules as amphiphilic peptide also show very high tendency to be self-assembled. Again, the self-assembled characters of the molecules are highly dependent on the sequence of amino acids in the peptides. It has been found that Phe-Phe (FF) sequence in the short peptide chain is highly potent for the formation of superstructures.

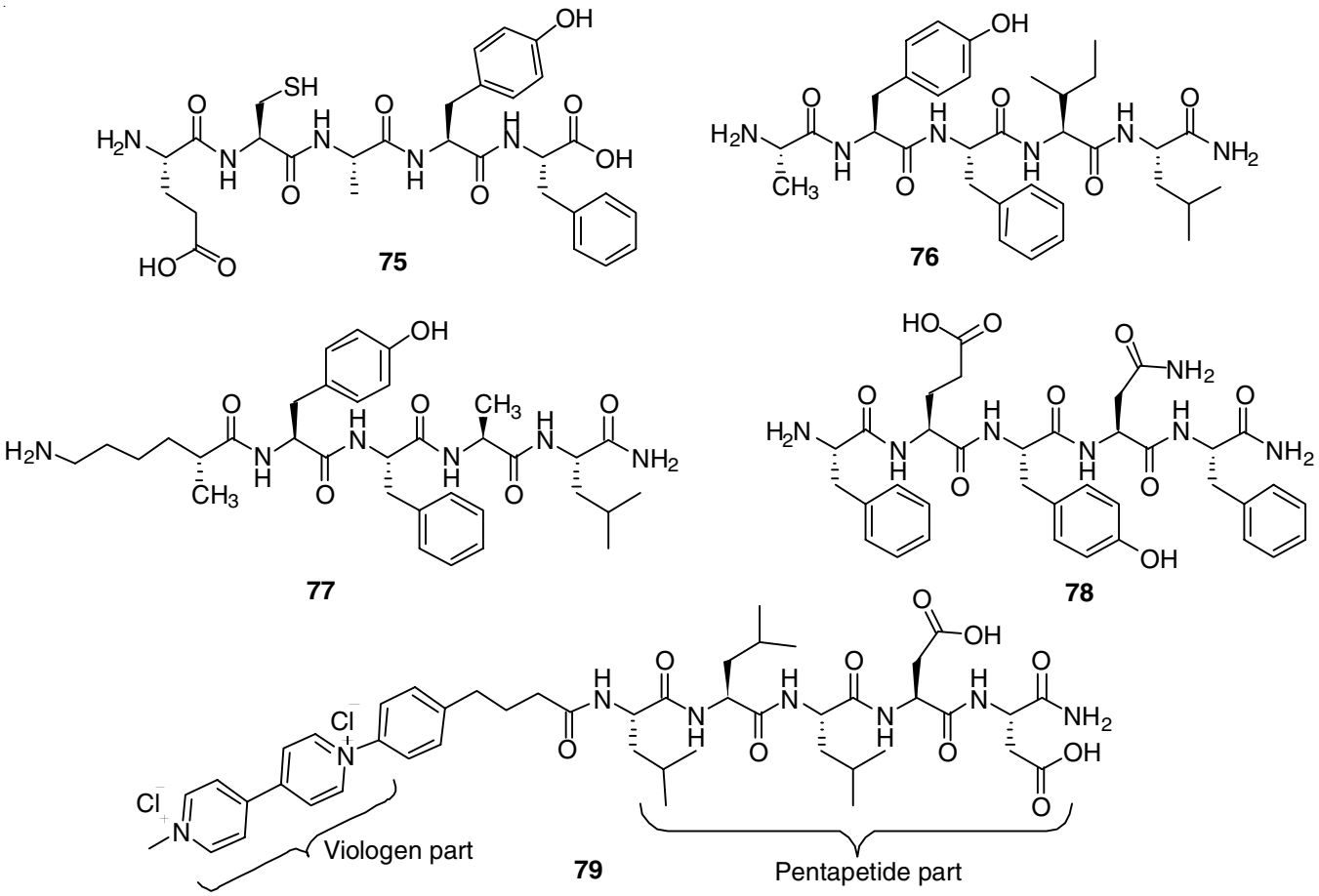

Fig. 14. Chemical structures of pentapeptides and pentapeptides derivatives **75**-**79**

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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