International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 402-413 www.ijprajournal.com ISSN: 2249-7781

Stapled Peptide as Drug Target

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Date of Submission: 1-11-2020 Date of Acceptance: 15-11-2020

ABSTRACT: Proteins, the building blocks of life, play a pivotal role in all the aspects of the cellular systems, from structural stabilization to the transport of nutrients, ions and small molecules, from immune defence to enzymatic activities.'Stapled peptides," consist of two amino acids (the most basic building block of peptides/proteins) chemically 'stapled' together in a way that mimics the binding of more complex proteins. A stapled peptide is one that has been chemically tweaked to maintain its unique shape.The need for introducing short peptides that regulate the important biological process in various types of diseases, have offered great potential as a new class of therapeutic candidates.undruggable therapeutic targets include those protein–protein interactions in which alpha-helices are required in lock-and-key-type mechanisms, an approach is to design alpha-helical peptides that have structural and functional properties that enable them to penetrate into the cell, bind to the therapeutic target, and modulate the biological pathway.

Keywords: Stapled Peptides,Protein-Protein Interaction,α-helix,Miniproteins

I. INTRODUCTION

Proteins that engage in intracellular interactions with other proteins are widely considered among the most biologically appealing yet chemically intractable targets for drug discovery. The critical interaction surfaces of these proteins typically lack the deep hydrophobic involutions that enable potent, selective targeting by small organic molecules, and their localization within the cell puts them beyond the reach of protein therapeutics.Considerable interest has therefore arisen in next-generation targeting molecules that combine the broad target recognition capabilities of protein therapeutics with the robust cell-penetrating ability of small molecules[(1)]. 'Stapled peptides," consist of two amino acids (the most basic building block of peptides/proteins) chemically 'stapled' together in a way that mimics the binding of more complex proteins. A stapled peptide is one that has been chemically tweaked to maintain its unique shape (in this case, a conformation called the alpha-helix, which looks like a curly ribbon). Without that shape, it could be blasted apart by enzymes, and wouldn't be as effective as a molecular switch. Too many protein-based drugs don't retain that alpha-helix shape and don't work. The stapled peptide retains its shape, and becomes biological stable, much more so that bigger proteins. It essentially merges the advantages of small molecules (evading the immune system, easily penetrating cell membranes and other defenses) with large biological molecules (acting more like 'real' biomolecules, more targeted treatments).[(2)]

One type that has shown promise in early-stage studies is hydrocarbon-stapled α-helical peptides, a novel class of synthetic miniproteins locked into their bioactive α-helical fold through the site-specific introduction of a chemical brace, an all-hydrocarbon staple. Stapled peptides are a nascent class of peptides that use stabilization technology to enhance potency and cell permeability to address pharmacological limitations of small molecules and existing biologics in intracellular protein–protein interactions. Stapled peptides are a nascent class of peptides that use stabilization technology to enhance potency and cell permeability to address pharmacological limitations of small molecules and

existing biologics in intracellular protein–protein interactions. The need for introducing short peptides that regulate the important biological process in various types of diseases, have offered great potential as a new class of therapeutic candidates. However, when isolated and introduced into an aqueous solution, peptide helices are highly susceptible to conformational changes. They are easily degraded by proteolysis and have difficulty in intact cell penetration, which often leads to a reduction in biological activity and thus diminishes therapeutic benefit[3].

This challenge can be overcome by chemically locking the conformational structure of a peptide which in turn mimics the molecular structures that are typically found at the interface of protein-protein interactions. When locked into this stable configuration, constraint peptides are able to penetrate cells efficiently and can exert their effects on intracellular protein targets. The large surface area of the peptides gives them advantages over small molecules in their ability to disrupt specific signaling pathways by inhibiting targeted protein-protein interactions.This review focuses on the successful design and evaluation of potent stapled peptide interactions that facilitate the broad application of this technology to intractable targets of both basic biologic interest and potential therapeutic value.[4]

STAPLED PEPTIDES

A peptide is simple a molecule that contains in it more than one amino acid. Peptides are smaller than proteins. If they are small enough to be synthesized, usually less than 50 molecules, they are by definition a peptide.A new technique to staple peptides to preserve their shape betterand help prevent them from transformation. The peptide stapling has the potential to have a positive benefit because the strong bond can disrupt protein to protein interactions involved in many biological areas, including the build up of stress and the death of cells.

Naturally, peptides are transforming between various arrangements. They generally shift between helices, sheet coil, and random coil. The protein stapling technique it is possible to staples the peptides together to prevent them from shifting forms. Peptides in a helix form enter cells easier than in other forms. The technique has the potential of also working of large chains of amino acids, proteins, which could add various new health benefits.[5]

Stapled peptides are a nascent class of peptides that use stabilization technology to enhance potency and cell permeability to address pharmacological limitations of small molecules and existing biologics in intracellular protein–protein interactions. Although small molecules are able to penetrate cells, the large binding surfaces for intracellular protein–protein interactions often make small-molecule modulators ineffective. Peptides and proteins have the size and functionality to effectively modulate intracellular protein–protein interactions, but do not permeate cells and, therefore, re used to modulate extracellular targets . Stapled peptides seek to resolve those problems. Because many undruggable therapeutic targets include those protein–protein interactions in which alpha-helices are required in lock-and-key-type mechanisms, an approach is to design alpha-helical peptides that have structural and functional properties that enable them to penetrate into the cell, bind to the therapeutic target, and modulate the biological pathway.

The Stapled Peptide Therapeutics are endowed with unique and beneficial drug-like properties that solve critical problems previously plaguing the peptide class of drugs. Stapled Peptides also retain the specificity and natural multitarget recognition capabilities of therapeutic proteins with very few limitations in their ability to address extracellular and intracellular targets [3].

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➢ **Stapled Peptides Have Benefits Versus Other Important Drug Classes**

Drug Properties	Small Molecules	Biologics	Peptides	Stapled Peptides
Cell penetration	++	--	--	++
Specificity	+	++	++	++
Stability	++	++	--	++
Target Space Opportunity	~10%	~10%	<10%	Large
Cost of manufacturing	$	$$$$	$$	$$
Concept to Drug (time)	⊕⊕⊕⊕	⊕	⊕⊕	⊕

SYNTHESIS OF STAPLED PEPTIDES

Protein–protein interactions that have been successfully disrupted by stapled peptides in vivo are characterized by a ligand-target pair. The ligand possesses an a-helical motif, borne on a protein or (better yet a) peptide, that docks into a shallow cleft on the surface of the target. Stapled peptide inhibitors represent "dominant-negative" versions of this docking helix optimized through synthetic modification (i.e., stapling) and sequence alteration to penetrate cells and compete effectively with the intracellular version of the ligand protein[6].

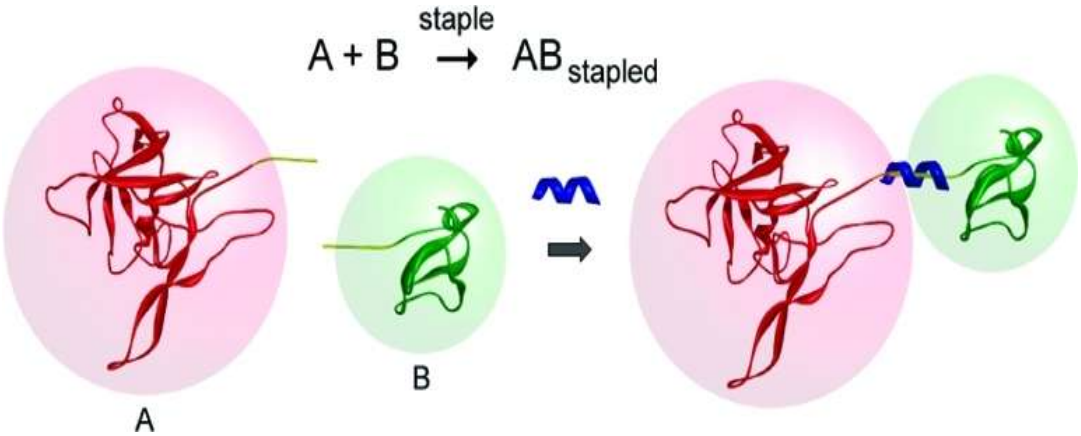

Fig: Schematic of protein stapling and design of staples.

1.1 Design of stapled peptides

When designing a stapled peptide, it is imperative to position the cross-linking amino acids such that the intended contact interface with the target protein(s) remains intact. For this reason, the design process is simplified by both atomic-resolution structures of the protein–protein interaction and mutagenesis data for residues at or near the binding interface. Other successful applications of stapled peptide inhibitors have to date also benefited from the use of high-resolution structures to direct the placement of the staple and, ultimately, the identification of the most potent inhibitor. If a structure is not available for a system of interest, but there is reason to believe that the ligand is α-helical when bound to the target, then other information such as Ala-scanning or residue conservation can be used as a basis for positionally biasing a stapling approach. In the absence of such information, it may be necessary to synthesize and screen a panel of stapled peptides encompassing most or all candidate stapling positions. A high-resolution structure of an approximately 60-amino acid α-helical portion of the MAML transcriptional coactivator protein bound in a cleft at the interface of CSL and the NOTCH oncoprotein guided the structure-based rational design of a potent stapled peptide inhibitor of NOTCH function . As it was already known that the recombinant α-helical MAML domain could act as a dominant-negative inhibitor of NOTCH-dependent transcriptional activation , constrained into an α-helical structure

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by all-hydrocarbon stapling, could produce a cell-penetrating antagonist of the NOTCH pathway. Due to the fact that the α-helical MAML peptide spans approximately 60 amino acids, there were many potential positions into which crosslinking amino acids could have been incorporated without disrupting critical residues at the protein–protein interface. Examining the NOTCH–CSL–MAML ternary complex, it was possible to hypothesize which amino acids in MAML were likely to be dispensable for binding to the NOTCH–CSL binary complex . The MAML peptide residues that contact neither NOTCH nor CSL were mapped onto the linear sequence of the MAML peptide in order to identify pairs of residues with the correct relative spacing such that they could be replaced to form one of the three types of all-hydrocarbon staples. It is also worth noting that a high-resolution structure can illuminate positions at which introduction of a rigid α-helical structure might be deleterious toward binding. The structure of the ternary NOTCH–CSL–MAML complex revealed the presence of a significant kink in the center of the MAML peptide at serine 46 and proline 47, two helix-destabilizing residues. A staple that spans this region might be expected to induce a more canonical α-helical structure than what promotes optimal binding, and the placement of a staple at this position would be expected to have a negative effect on binding. Upon analysis of the atomic-resolution structure of the NOTCH–CSL–MAML complex, stapling positions anticipated to be compatible with maintenance of essential binding contacts were identified. Subsequently, the 60-mer MAML peptide was divided into smaller segments of approximately 12–20 amino acids, and a panel of analogs bearing all-hydrocarbon staples was synthesized and tested for inhibition of complex assembly. Ultimately, the i, iþ4 stapled peptide SAHM1, which spanned 16 residues in the N-terminal portion of the 60-mer MAML peptide andfeatured the replacement of glutamate 28 and arginine 32 with S5 crosslinking amino acids, was identified as a potent antagonist of complex assembly.

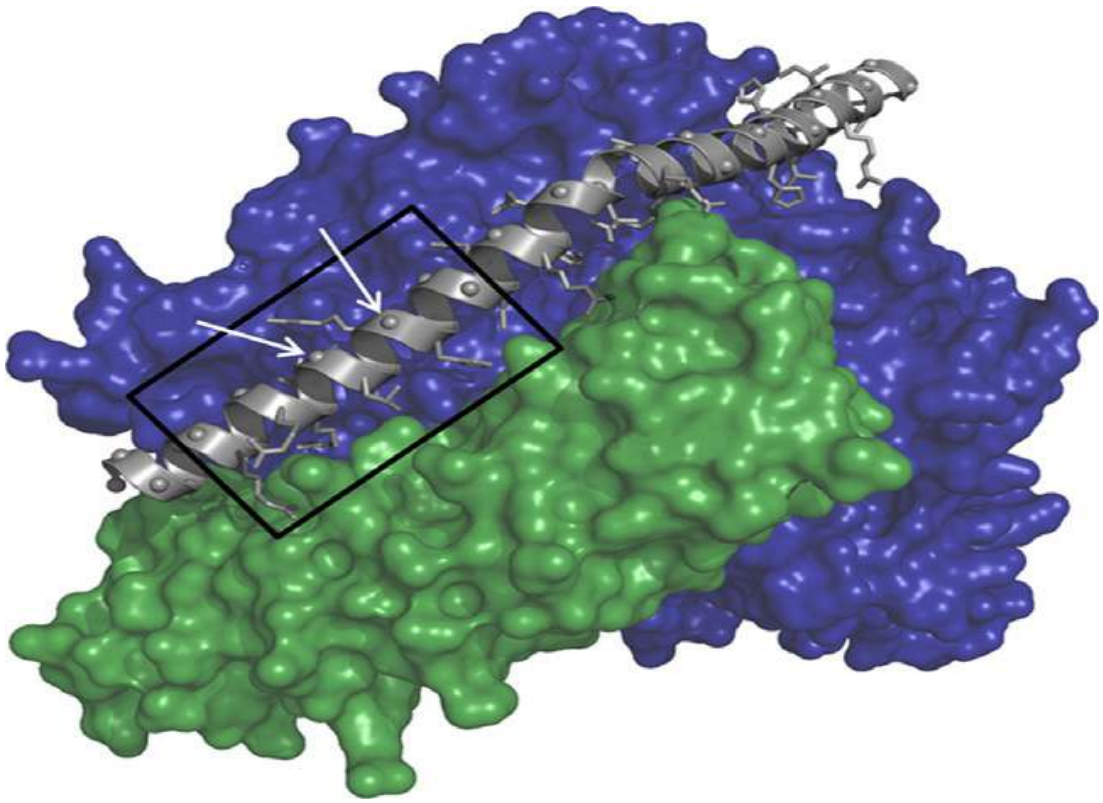

Fig: The design of stapled peptides targeting the NOTCH/CSL binary complex.

After identifying a stapled peptide that interacts with the target, an important parallel aspect is to design a negative control stapled peptide that can be used to confirm target-specific effects in vitro and in vivo. The design of a negative control stapled peptide is highly context

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dependent, and thus it is difficult here to provide a general design proscription. In some cases, residues previously validated as important for target interaction can be mutated nonconservatively to produce a negative control stapled peptide .If residues in the parent sequence of the stapled peptide have not been explicitly validated as determinants of binding specificity, structure-based design can be utilized to introduce mutations that would be expected to disrupt key interactions. During the design of a negative control, it is important to consider the effects that any engineered mutations might have on other properties of the stapled peptide.

1.2 Stapled Peptide Synthesis

Stapled peptides are typically synthesized by SPPS using amino acids With acid-labile side chain protecting groups and a base labile fluorenylmethoxycarbonyl (Fmoc) protecting group on the back bone amine . N-a-Fmoc-protected amino acids are often offered with a choice of side chain protecting groups for standard SPPS of stapled peptides, the side chain protecting groups indicated. Reagents are typically purchased from Sigma–Aldrich or EMD Chemicals and used as received. Due to the use of liquid ammonia and sodium or lithium metal in a dangerous dissolving metal reduction step,it is recommended that the Fmoc-protected α-methyl, α-alkenyl crosslinking amino acids are synthesized using the Ala-Ni(II)–BPB complex method Rink Amide MBHA resin (100–200 mesh, loading 0.4–0.8mmol/g) is used as a solid support for the synthesis of stapled peptides with an amidatedC-terminus.

A VacManOLaboratory Vacuum Manifold (Promega) or similar multiport apparatus can be used for the manual parallel synthesis of a panel of stapled peptides. The waste reservoir is connected to the house vacuum, and each port that will be used for peptide synthesis is fitted with a solvent resistant 3-way stopcock (Bio-Rad) connected to a nitrogen stream and a disposable polypropylene chromatography column (Bio-Rad). When preparing a panel of stapled peptides for initial screening, a synthetic scale in the range of 10–25µmol typically yields a sufficient amount of stapled peptide for biophysical characterization, in vitro target interaction assays, and evaluation of cell penetration and efficacy in cell-based activity assays. This synthetic scale can be accommodated using a chromatography column with a 2mL bed volume; chromatography columns with different bed volumes can be employed if necessitated by the synthetic scale.

For a 25µmol scale synthesis, 0.038g of Rink Amide MBHA resin is added to each chromatography column. Approximately 1mL of N-methyl- 2-pyrrolidone (NMP) is added and the resin is bubbled under nitrogen for at least 30min to achieve thorough swelling of the resin.The peptide chain is then elongated from C- to N-terminus by a repeating cycle of N-α-Fmoc deprotection and subsequent coupling of an N-α-Fmoc-protected amino acid to the nascent peptide chain . Extensive washing of the resin is performed after each instance of deprotection and coupling, and all steps are performed with nitrogen bubbling to ensure equal contact of all beads with the reaction solution. A detailed description of one cycle of deprotection and coupling is described below.

(1) Deprotect N-α-Fmoc by 3×10min treatments with 1mL of 25% (v/v) piperidine in NMP.

(2) Wash resin with 5×1min treatments with 1mL of NMP.

(3) Couple activated amino acid to the deprotected amine by one treatment with the following solution (activated amino acid solution is premixed prior to addition to the reaction vessel)

approximate coupling time is based on the guidelines below

- **Activated amino acid solution (natural amino acids)**

0.375mL of 0.4MN-α-Fmoc-protected amino acid in NMP (6equiv)

0.375mL of 0.38M HCTU coupling reagentin NMP (5.7equiv)

0.052mL of DIPEA (N,N-diisopropylethylamine) (12equiv)

- **Activated amino acid solution (cross-linking amino acids)**

0.250mL of 0.4MN-a-Fmoc-protected amino acid in NMP (4equiv)

0.250mL of 0.38M HCTU coupling reagent in NMP (3.8equiv)

0.035mL of DIPEA (8equiv)

- **Coupling time**

Natural amino acids (not including sterically hindered aminoacids):45min.

Sterically hindered natural amino acids (His, Ile, Pro, Thr, Trp, Val):60min

Cross-linking amino acids: 60min

(4) Wash resin with 5×1min treatments with 1mL of NMP.

(5) Repeat steps 1×4 for each amino acid.

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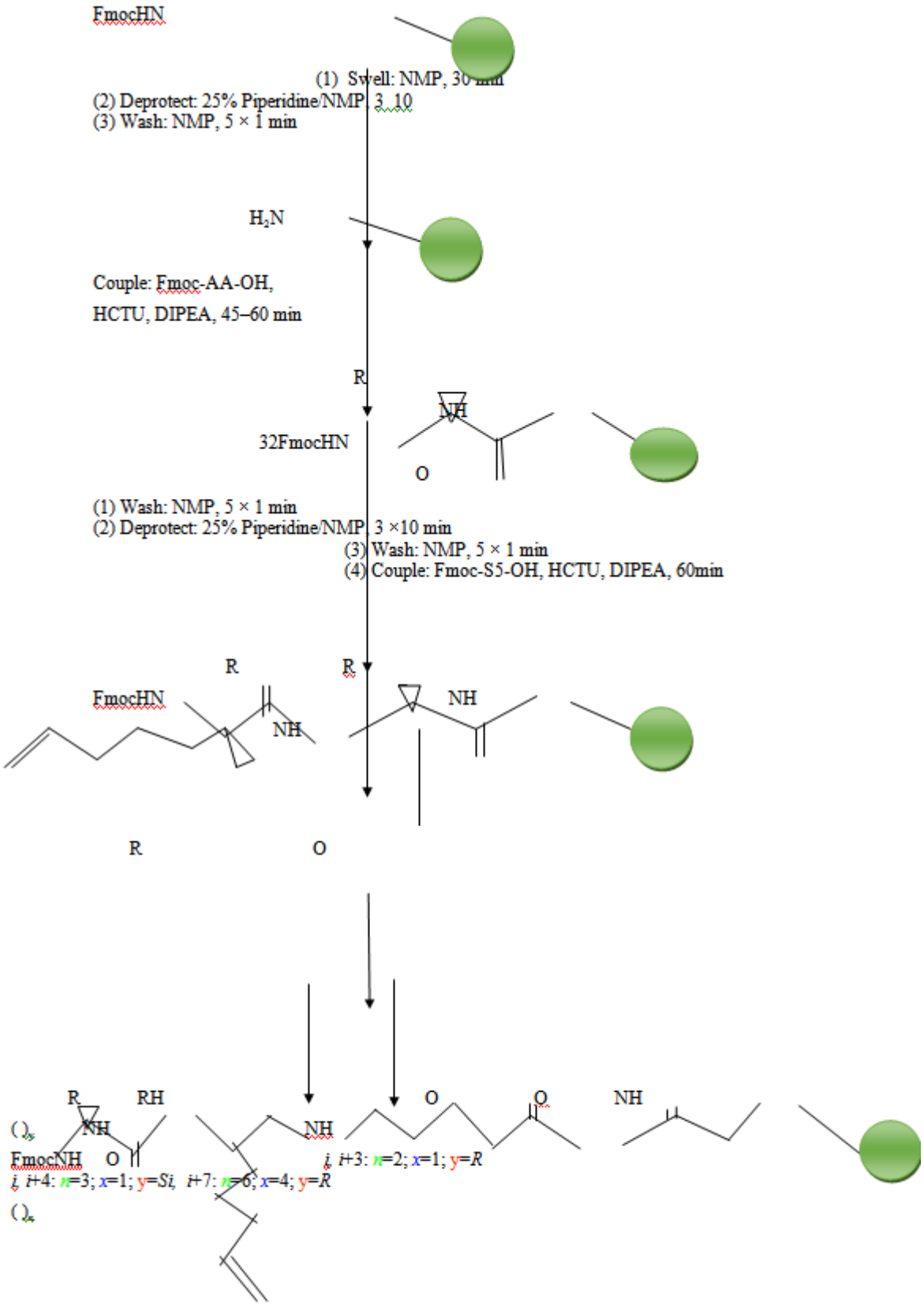

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Fig: Fmoc-based solid-phase peptide synthesis (SPPS) of all-hydrocarbon stapled peptides. Rink Amide MBHA resin is swollen in NMP and the Fmoc protecting group is removed by treatment with 25% piperidine in NMP. An Fmoc amino acid is activated by HCTU and coupled to the resin in the presence of DIPEA. The cycle of Fmoc deprotection followed by coupling of an activated amino acid is repeated to elongate the peptide. a-Methyl,a-alkenylglycine cross-linking amino acids are incorporated as shown to produce one of the three types of stapled peptides.

Peptide synthesis can be paused overnight after completion of a coupling step. The resin is washed with NMP as previously described, and then the following procedure is used to dry and shrink the resin:
(1) Wash resin with 3×1min treatments with 1mL of dichloromethane (DCM).
(2) Add 1mL methanol to the resin and bubble to dryness with N2(g)[7].

II. THERAPEUTIC ACIONS OF STAPLED PEPTIDES

2.1 Anti apoptotic activity

A major problem with many cancer chemotherapeutic drugs is their lack of selectivity. They attack and kill not only the cancer target of interest, but in many cases also normal cells in tissues that are vital for cancer patient survival. Drugs that target cellular functions that are essential specifically for cancer cell survival should thus be of benefit to the cancer patient undergoing chemotherapy.Cancer cells have adapted numerous strategies to avoid apoptosis, or programmed cell death. The BCL-2 family contains anti-apoptotic and pro-apoptotic proteins. The anti-apoptotic BCL-2 family proteins have been linked to the survival of pathogenic cells that include cancer cells. RNA inhibition has been one approach to blocking anti-apoptotic BCL-2 and hence cancer cell survival.An alternative approach that has emerged to modulate, either positively or negatively, the activity of apoptotic signaling proteins is the use of stapled peptides. Peptides derived from native protein sequences are unfolded, and tend to shift conformations between helices, sheets and random coils, because they have been removed from the stabilizing influences of the protein milieu in which they naturally reside. The regularity of the α helical backbone conformation in the peptide can be maintained by introducing non-natural amino acids into the peptide sequence; the non-natural amino acids are incorporated on adjacent turns of the α- helix. A covalent bond (“staple”) between non- natural amino acid residues containing terminal double bonds is formed by a ruthenium-catalyzed metathesis reaction that closes the ring, tethers the two non-natural amino acids and stabilizes the peptide in the α- helical configuration. The use of alkyl tethers to produce an all-hydrocarbon chain linking the non- natural amino acids greatly stabilized the helical peptide, in comparison to non-hydrocarbon substituents. The use of an all-hydrocarbon cross-linker was a key development that greatly improved cell uptake of the peptides, as well as their resistance to protease degradation and their effectiveness in modifying cancer cell survival. These are all qualities that are highly desirable in a chemotherapeutic agent[8].

The efficacy of peptide therapeutics could be greatly improved using peptide stabilization approaches such as peptide stapling. Stapled peptides have several appealing features as agents for cancer chemotherapy. First, they can very specifically bind to and interfere with the function of anti-apoptotic proteins in the cell. A variety of drugs are used to alter apoptosis in cancer, but in many instancesthey are not highly specific for a single target, as the stapled peptides appear to be. Second, peptides are relatively inexpensive, with the caveat that the addition of hydrophobic staples would boost the cost. Third, peptides are more cell permeable than antibodies and some chemotherapeutic drugs.

The major shortcoming of cancer chemotherapy is development of resistance, and there is little or no information about whether patients will become resistant to SAHBs. Chemotherapy regimens based on stapled peptides that bind to cancer targets have great potential to benefit the cancer patient, because these reagents exhibit superior specificity in interacting with the target, and can be selected to either augment or inhibit target activity in a manner favorable to the patient[9].

2.2Targeting neuro endocrinal cells

Precise cellular targeting of macromolecular cargos has important biotechnological and medical implications. Using ‘protein stapling’ method, the proteolytic domain of botulinum neurotoxin type A (BoNT/A) linked to a selection of ligands to target neuroendocrine tumor cells.The botulinum proteolytic domain was chosen because of its well-known potency to block

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the release of neurotransmitters and hormones. Among nine tested stapled ligands, the epidermal growth factor was able to deliver the botulinum enzyme into pheochromocytoma PC12 and insulinoma Min6 cells; ciliary neurotrophic factor was effective on neuroblastoma SH-SY5Y and Neuro2A cells, whereas corticotropin-releasing hormone was active on pituitary AtT-20 cells and the two neuroblastoma cell lines. In neuronal cultures, the epidermal growth factor and ciliary neurotrophic factor-directed botulinum enzyme targeted distinct subsets of neurons whereas the whole native neurotoxin targeted the cortical neurons indiscriminately. At nanomolar concentrations, the retargeted botulinum molecules were able to inhibit stimulated release of hormones from tested cell lines suggesting their application for treatments of neuroendocrine disorders[10].

2.3Binding to estrogen receptors.

Estrogen receptor (ER), a member of the steroid hormone receptor class of NRs, regulates reproduction and additionally plays a critical regulatory role in a broad range of physiological systems including, amongst others, the central nervous system, bone density, and immunity. As such ER has been targeted, with much success, as a point of intervention in a number of disease states, principally in breast and endometrial cancers, and osteoporosis. Two ER isoforms exist, ERα and ERβ, which share the characteristic domain organization of NRs, namely a variable N-terminal transactivation (AF1) domain, the well conserved DNA binding domain and a Cterminal ligand binding domain (LBD). Estrogens bind to the LBD and induce a conformational change in the receptor that promotes homodimerisation and the subsequent binding to specific promoter DNA sequences in target genes modulating gene expression. However, an additional group of binding partners exist, the co-regulatory proteins which are recruited to this complex. ER requires the direct binding of a co-activator protein for ligand dependent signaling to occur.

Recently a new class of stabilized helix peptidomimetic agent has received significant attention, the stapled peptide18. Stapled peptides contain an all-hydrocarbon link (the ‘staple’) between successive turns of a peptide α-helix. The key to this approach is the addition of two un-natural amino acids containing olefinbearing tethers which covalently link the i, i+3, i, i+4 or i, i+7 positions in a peptide (corresponding to amino acids separated by one or two turns of the desired helix)19. These un-natural amino acids also have a α-methyl group which further stabilizes the α-helix conformation[11].

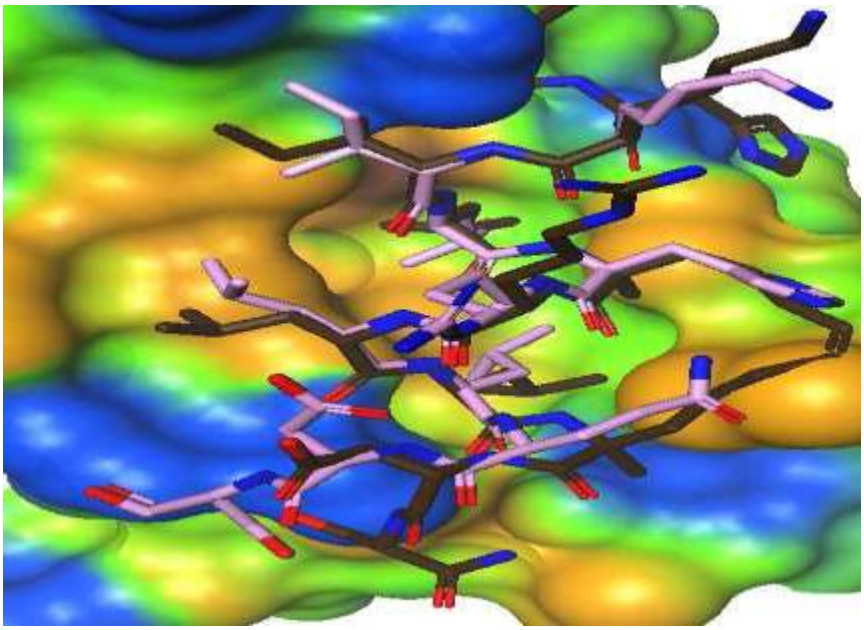

Fig:stapled estrogen receptor.

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2.4 Targeting androgen receptors

The androgen receptor engages in a ligand-dependent interaction with short a-helical regions from a variety of transcription coactivator proteins. Nearly one hundred stapled peptides bearing the FxxLF or a related motif critical for mediating binding to the androgen receptor were designed and screened for the ability to bind to the androgen receptor in vitro and inhibit the proliferation of an AR-dependent prostate cancer cell line. A subset of the stapled peptides bound to the androgen receptor with low nanomolar affinities and lead compounds that displayed potential in the cellular proliferation assay were identified[12].

2.5 Targeting tumours

Cancer biologists consider many cancer-related proteins as 'undruggable' because they resist treatments from traditional drugs. Chemical biologists have realized that interfering with the crucial p53:Mdm2 interaction is a viable cancer treatment strategy; however, it is no easy task to find drug compounds that permeate cells and survive to reach their targets. Peptide α-helices that adhere tightly to Mdm2's p53 binding domain make attractive drug candidates because of their low toxicity and site-specific potency. Unfortunately, native peptide helices tend to unravel and decompose inside cells.Stapled peptides can activate the tumor-suppressing protein p53 inside cells by disrupting[13].

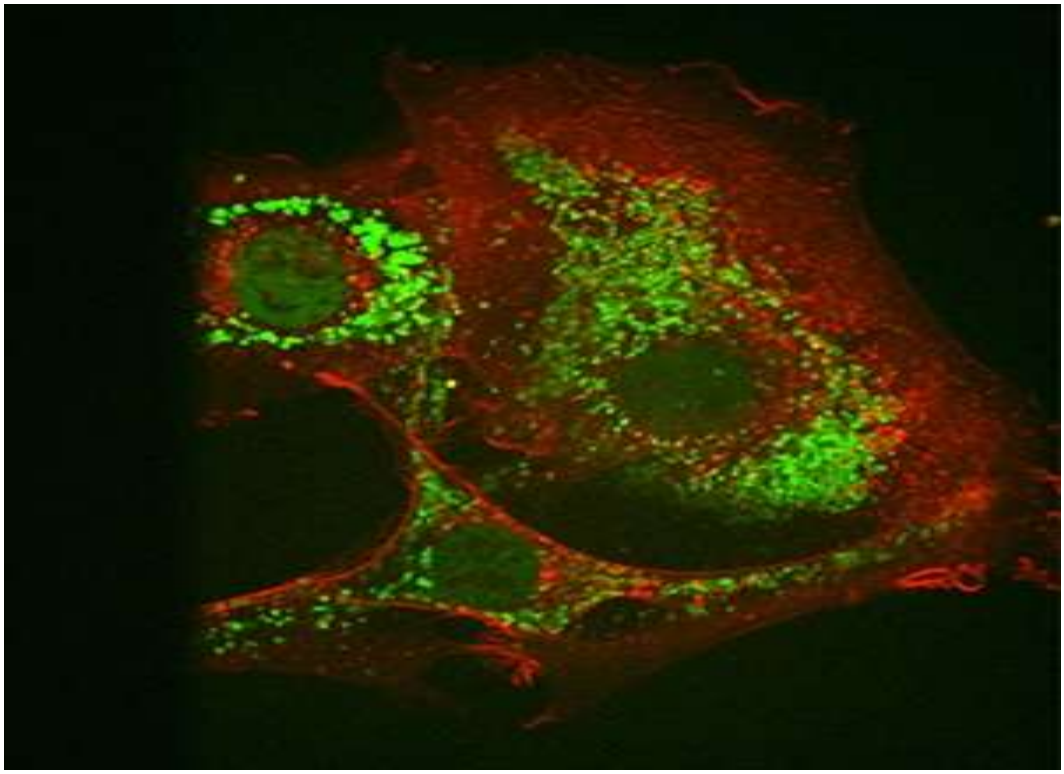

Fig: microscopy image of a stapled peptide (fluorescently labeled green) being incorporated into a live murine T22 cell array. The peptide will activate the p53 tumor suppressor protein. Cell membrane is labeled in red.

In leukemia stapled peptides inhibit the function of NOTCH protein. Stapled peptides was able to stop Notch from working and to reduce the growth of leukaemia. The technique may lead to the development of new drugs to treat this type of leukaemia (called T-ALL), and to potential ways of using stapled peptides in other areas of research. Inside the cell, a protein called MAML1 binds to a complex of proteins that contains the NOTCH1 transcription factor. A fragment of the MAML1 protein (called dnMAML1) can block the action of NOTCH1 in T-ALL leukaemia cells, stopping them from dividing[14].

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Fig:T-ALLleukaemia cells

2.6 Targeting HIV-1 entry and assembly

The mechanism of action and antiviral activity of a series of $i,i+7$ stapled peptides derived from CAI. We show that this class of stapled peptides inhibit both assembly of infectious HIV-1 and its entry; thereby acting as dual-targeted inhibitors. NMR studies indicate that these stapled peptides strongly bind to HIV-1 CA, although not all of them significantly perturb in vitroCA assembly. In addition, the ability of these peptides to inhibit virus assembly appears to be dependent on the efficiency of cell penetration. Resistance studies to delineate the target and mechanism of inhibition suggested the involvement of the gp120 V3 loop, a region of gp120 critical for Env-mediated membrane fusion and viral infection . Biophysical studies using isothermal titration calorimetry (ITC) confirmed that these peptides bind strongly to the V3 loop. The critical findings detailed here illustrate dual inhibition of HIV-1 assembly and viral entry through specific targeting of HIV-1 CA and gp120, respectively[15].

III. NOVEL STAPLED PEPTIDE NANOPARTICLE COMBINATION PREVENTS RSV INFECTION

Researchers have developed novel double-stapled peptides that inhibit RSV(Respiratory Syncytial Virus) in cells and in mice. The team also showed that this peptide's capacity to block infection was significantly boosted when delivered to the lungs by miniscule, biodegradable particles known as nanoparticles.double-stapled peptide interference targeting the virus fusion protein can be administered in the form of a nasal drop or spray. The treatment suppressed viral entry and reproduction, including spread from nose to lungs, providing substantial protection from infection when administered several days before viral exposure.Peptide stapling restores the natural helical shape, which also inhibits proteolysis, providing a new opportunity to take advantage of a well-validated mechanism of action to thwart viruses like RSV that otherwise lack drugs for preventing or treating infection[16].

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IV. FUTURE DEVELOPMENTS OF STAPLED PEPTIDES

Advanced research in stapled peptides in both drug design and peptide synthesis. Researchers at the New York Structural Biology Center reported on high-resolution nuclear magnetic resonance techniques with dynamic light-scattering to characterize a family of hydrocarbon-stapled peptides with known inhibitory activity against the HIV-1 capsid assembly to evaluate the various factors that modulate activity. The researchers reported that helical peptides share a common binding motif but differ in charge, the length and position of the staple. The research showed that the peptides share a propensity to self-associate into organised polymeric structures mediated predominantly by hydrophobic interactions between the olefinic chain and the aromatic side-chains from the peptide. The researchers also detailed the structural significance of the length and position of the staple and of the olefinic bond isomerization in stabilizing the helical conformation of the peptides as potential factors influencing polymerisation .

The use of hydrocarbon double-stapling to remedy the proteolytic instability of a lengthy peptide. Specifically, the researchers applied the stapled approach to Fuzeon (enfuvirtide), a 36-amino-acid peptide that inhibits human immunodeficiency virus Type 1 (HIV-1) infection by targeting the viral fusion apparatus[(15)].

Enfuvirtide is used as a salvage treatment option because of poor in vivo stability and poor oral bioavailability. To address the proteolytic shortcomings of long peptides as therapeutics, the researchers studied the biophysical, biological and pharmacological impact of inserting all-hydrocarbon staples into the drug. The researchers found that the peptide double-stapling created protease resistance and improved pharmacokinetic properties, including oral absorption. The hydrocarbon staples created a "proteolytic shield" by reinforcing the overall alpha-helical structure, which slowed the kinetics of proteolysis and also created a complete blockade of peptide cleavage at the constrained sites in the immediate vicinity of the staple. The researchers noted the potential of double-stapling to other lengthy peptide-based drugs[(3)].

Stabilized BimBH3 peptides (BimSAHB), which had reduced affinity for their targets, the pro-survival Bcl-2 proteins .The researchers attributed the loss in affinity to disruption of a network of stabilizing intramolecular interactions present in the bound state of the native peptide. They suggested that altering the network may compromise binding affinity, as in the case of the BimBH3 stapled peptide in their study. They also said that cells exposed to these peptides do not readily undergo apoptosis, which indicates that BimSAHB is not inherently cell permeable[(8)].

V. CONCLUSION

Stapled peptides refers to a computational drug design technique that may create a whole new class of drugs by being able to more effectively target substances within cells an increase the number of proteins which can be targeted. Stapled peptides are generated through synthetic enhancement of a 3-D alpha-helix protein segment which hydrocarbon bonds to make proteins more rigid and able to penitrate cell walls.

Stapled peptide could allow a wider range of proteins to be used in drug – targeting.They currently in clinical trials for the inhibition of a BCL-2 family protein, oncogene MCL-1, using an exclusive inhibitor,the MCL-1BH3helix,which could unblock caspase-dependent apoptosis in cancer cells.The double-stapled peptide interference targeting the virus fusion protein can be administered in the form of a nasal drop or spray. The treatment suppressed viral entry and reproduction, including spread from nose to lungs, providing substantial protection from infection.

Stapled α−helical peptides have emerged as a promising new modality for a wide range of therapeutic targets. The stapled peptide approach is one step toward being able to produce properly configured drug molecules that can bind with appropriate proteins. Stapled peptides are starting to get a lot of attention for drug discovery and development by targeting protein-protein interactions.

New drug formulations based on sapledeptides are uder clinical studies.

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