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PEPTIDES AS PHARMACEUTICAL LEADS: A MECHANISTIC BASED EXPLORATION THROUGH MOLECULAR MODELING AND DOCKING STUDIES

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ABSTRACT

In this study the pharmaceutical potential of naturally occurring antimicrobial peptides has been explored taking a mechanistic approach. The mechanistic aspects of their antifungal property and anti-HIV property have been studied in detail. The antifungal nature has been explained at the molecular level through their ion binding capacity. The antimicrobial peptides can bind to the HIV viral template by interactions other than base-pair base-pair type. A tight complex formation has been observed in molecular modeling and docking calculations. These fascinating properties of antimicrobial peptides have led to their exploration as pharmaceutical leads repeatedly but these compounds never gained success in the drug market due to their poor pharmacokinetics. Another added benefit of peptide leads is their capacity to curb microbial resistance issues. With these perspectives this study also investigates the ADME properties of these compounds and design of peptidomimetic leads with artificial backbone to enhance druggability. The importance of molecular modeling and docking studies in structural modification of antimicrobial peptides, design of peptidomimetic compounds with artificial backbone is also discussed. This work may guide experimentalists in the field to come up with non toxic, robust drugs for intertwined approach to deal with two fatal diseases HIV and internal fungal infection.

KEYWORDS: antimicrobial peptide, anti-HIV, ion carriage, intermolecular interaction, docking, internal fungal infection, intertwined approach.

INTRODUCTION

Antibiotics constitute an important class of drugs worldwide. Needless to highlight the role of these drugs in treating and controlling the spread of bacterial infections and related diseases worldwide. The success story of antibiotics has now been alarmingly dampened by the rise in occurrences of antibiotic resistant strains of bacteria which robustly continue to spread and stand unabated. When an antibiotic is unable to control the spread of certain strain of bacteria it is called antibiotic resistant. Such incidences are now increasing and have gone up to the level of multidrug resistance as well as totally drug resistant even for fatal disease causing bacteria like Mycobacterium tuberculosis.[1] Antibiotics must be used to treat severe microbial infections. The unnecessary over usage of antibiotics also contributes towards development of bacterial resistance. Therefore, it is only logical to understand ways to circumvent resistance issues as opposed to discovery of new antibiotics.[2] How do bacteria acquire antimicrobial resistance (AMR), that is, how do they acquire the ability to resist the effect of an antibiotic used against them? They start developing modifying enzymes which alter the antibiotic in a way that it becomes ineffective.[3] Several aminoglycoside antibiotic modifying enzymes are known, for example: O-phosphoryl transferases, N-acetyl transferases, O-adenyl transferases etc. These enzymes are commonly found in bacterial pathogens.[4-5] With the advent of newer, more resistant species of pathogens, research in this area has taken a new direction.

Naturally occurring antimicrobial peptides (AMPs) used by flora and fauna for self defense seemed a lucrative alternative for a number of reasons: i. Natural compounds are not easily modified. ii. Natural compounds may be less toxic and more biocompatible. iii. Naturally occurring cationic peptides assist in intracellular delivery. Hence, a lot of research work including isolation, synthesis and testing was carried out on these peptides[6-10] but very few peptides actually reached the commercialization stage due to their bioavailability problems.[11]

In this chapter we discuss the various properties possessed by these peptides and their immense potential as pharmaceutical leads. The chapter also discusses a mechanistic approach and its advantages towards design of peptidomimetic lead compounds. Computational probe of mechanistic aspects of AMPs is not an easy task due to the dimensionality of the problem and the degrees of freedom involved. In this chapter we describe a combination of ab initio molecular orbital calculations and molecular docking

studies for the development of peptidomimetic lead compounds to overcome drug resistance issues. The ab initio molecular orbital calculations being highly accurate help scan the conformational surface of AMPs. The docking tools have been employed to first understand mechanistic aspects and then aid in the design of mimetic compounds with predictably improved pharmacokinetic properties. Introduction of artificial side chains or alteration of peptidomimetic backbone is an impossible task without proper molecular modeling and docking tools. Structural changes were introduced and then docking tools were used to assess the impact of structural changes on mechanism and thus the therapeutic potential of designed peptidomimetic lead compound.

METHODOLOGY

In this study, we wish to understand at the molecular level how a peptide holds a metal ion non-covalently? What type of non-bonded interactions like hydrogen bonding, electrostatic interactions stabilize such a complex formation? To this effect, we have performed accurate quantum mechanical Hartree-Fock (HF) molecular orbital calculations.[12] All geometrical parameters were allowed to relax using Berny's gradient method.[13,14] The calculations were performed twice on each compound; once in absence of metal ion and then in presence of metal ion. Ab initio molecular orbital calculations have been performed at the Hartree-Fock (HF)/6-31G.[15] basis set utilizing Gaussion'09 software.[16] Since the geometrical parameters were allowed to relax, the peptide reorganizes in presence of metal ion to maximize interactions with it. The energy of this reorganized compound and metal ion were calculated separately to yield the interaction energy as follows.

$E_{int} = E_{complex} - (E_{peptide} + E_{ion})$

Energy required for reorganization of peptide in presence of metal ion to hold the ion efficiently has been calculated as.

$E_{reorganization} = E_{reorganized\ peptide} - E_{peptide\ without\ metal\ ion}$

The combination of both gives the overall stabilization of metal ion with the peptide.

Our study also explores the possibilities of some AMPs inhibiting the viral transcription process by interacting with the viral RNA template, through interactions other than base pair-base pair type. A combination of molecular modeling, docking and MMGBSA binding energy calculations have been performed to study inhibition of HIV viral replication process by small peptides. In addition, ab initio quantum mechanical intermolecular interaction

energy calculations have also been performed at the Hartree–Fock level on docked complexes to understand their relative stabilities. For peptide and template preparation, we have taken modeled structures which are based on solution NMR data. Modeled structures have been reported for several AMPs and are available in the Brookhaven protein databank. Choice of peptides was based on their antifungal activity and small size suitable for ab initio calculations. The best representative structure was taken from the ensemble in pdb file and prepared using Protein Preparation Wizard of Schrodinger software.[17] The primer binding site (PBS) of HIV viral single-stranded (ss) RNA was taken from pdb Id 4B3O[18] corresponding to the HIV-1RT ternary complex. The PBS of viral RNA, which acts as template in reverse transcription process, was then prepared utilizing the Protein Preparation Wizard of Schrödinger software.

To study the interaction of the chosen AMPs with the PBS of viral template and to judge their capability to block PBS, the peptide was now docked into the template choosing the entire PBS as target. A grid was placed at the center of the template. Standard precision flexible ligand docking with post-docking minimization was performed utilizing the Glide module[19] of Schrödinger software. Glide uses a series of hierarchical filters to search for possible locations of ligand in the active site region.

Glide uses a modified scoring function as compared to conventional molecular mechanics energy function for predicting binding affinity and rank-ordering the ligands in the database. The standard precision scoring function seeks to minimize false negatives and the extra precision imposes severe penalties for violating basic principles. The standard precision scoring function is defined in brief as.

G Score = 0.05 vdw + 0.15 Coul + Lipo + H Bond + Metal + Rewards + Rot B+ Site

Where vdw = van der Waals energy

Coul = Coulombic energy

Lipo = Lipophilic term derived from hydrophobic grid potential

H Bond = Hydrogen bonding term

Metal = Metal binding term

Rewards = Rewards and penalties that cover other terms than those explicitly mentioned like buried polar groups,

hydrophobic enclosure, amide twists etc.

Rot B = Penalty for freezing rotatable bonds

and Site = Polar but non-hydrogen-bonding interactions in the active site.

Best poses from standard precision docking were subjected to extra precision docking, which is designed to weed out poses with unfavorable interactions and give a better correlation between good poses and good scores. A maximum of 50 poses per ligand were subjected to postdocking minimization, out of which the 10 best poses were filtered. For the studied peptides, a maximum of three poses were generated in each case after post-docking minimization. Because docking has been performed to a stretch of ssRNA as opposed to the binding pocket of any rigid protein, it is prone to a certain amount of uncertainty as the grid placement can also not be authenticated. To endorse the extra precision docking results, we have performed accurate, large ab initio intermolecular interaction energy calculations on the best poses obtained. The best pose in each case sorted on Glide energy was then subjected to ab initio intermolecular interaction calculations at 6-31G basis set[15] and subsequently, combined molecular mechanics generalized born surface area (MMGBSA) binding energy evaluation with implicit solvent around the complex.

Ab initio molecular orbital calculations have been performed at the Hartree–Fock (HF)/6-31G[15] level utilizing Gaussian ’09 software.[16] The interaction energy between peptide and template has been calculated by the supermolecule approach without basis set superposition error (BSSE) as follows.

Interaction Energy $=E_{complex} - (E_{peptide} + E_{template})$

MMGBSA calculations have been performed using the Prime module of Schrödinger software[20] with Maestro interface. MMGBSA approach uses molecular mechanics OPLS 2005 force field coupled with a generalized Born surface area continuum solvent model for the prediction of solvation energies of complex and the two fragments.[21] Binding energies are then evaluated as follows.

$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{sol} + \Delta G_{SA}$

Where, ΔE_{MM} is the difference in minimized energies of complex and sum of energies of viral template and peptide ligand.

ΔG_{sol} is difference in solvation energies of complex and sum of solvation energies of viral template and peptide ligand.

ΔG_{SA} is the difference in surface area energies of complex and sum of surface area energies of viral template and peptide ligand.

Based on docking results peptidomimetic compound have been designed to enhance druggability features. To help understand druggability of designed compounds we have studied its pharmacodynamic and pharmacokinetic aspects which are referred to in brief as ADME properties for absorption, distribution, metabolism and excretion. ADME properties of these peptides have been estimated using Qik Prop module of Schrodinger software.[22] AMPs with good interaction energies were chosen and modified strategically to enhance druggability features to achieve lead compounds for the development of safer, non-toxic antimicrobial agents with anti-HIV activity.

RESULTS AND DISCUSSION

ANTIMICROBIAL PEPTIDES (AMPs)

The structure and properties of several naturally occurring AMPs (largely cationic) have been studied. Some of the important data are collected in table 1.[23-35]

It is clear from table 1 that most of the AMPs are cationic, non cyclic peptides. Commonly studied AMPs include chitinases from insects, anabaenolysins from cyanobacteria, scolopendins, lactobacillus strains and small peptides derived from amphibian skin and spider venoms. Their antibacterial, antifungal and anti-HIV activities have been measured.[36-64] However, the experimental data being diverse due to differences in targeted species, is hard to compare and correlate with theoretical findings. An attempt has been made in this work to correlate and analyze as best as possible. To be able to harness the properties of these peptides it is necessary to understand the underlying mechanism of action. In the next few sections, we discuss the mechanistic aspects of various properties of these peptides.

Table 1: Antimicrobial Peptides and their MICs.

Antimicrobial peptides	Charge on peptides	Natural Sources	Structural file (RCSB protein data bank)	Peptide sequence	Antifungal activity		Anti-HIV activity	Ref.
					MICs (µg/ml)	Target Species	HIV IC50 (µM)	
rTD-1	+5	*Rhesus macaque* (Old world monkeys)	2LYF	GFCRCLCRRGVCRCICTR	1	*C. albicans*	0.45-1.9	[23][24]
Tachyplesin I	+6	(Horseshoe crab)	1MA2	KWCFRVCYRGICYRRCR	3.1	*C. albicans*	>20	[25][26]
Protegrin2	+5	*Sus scrofa* (Wild boar)	2MUH	RGGRLCYCRRRFCVCV	3.57	*C. albicans*	12.8	[27] [28]
Indolicidin **Indolicidin variant** (CP-11)	+4 +6	*Bos taurus* (Cattle)	1G89 1QXQ	ILPWKWPWWPWRR ILKKWPWWPWRRK	3.8-30.5	*C. albicans*	47 -	[29][30]
Cm-p5	+2	*Cenchritis muricatus* (Sea snail)	2MP9	SRSELIVHQRLF	10	*C. albicans*	-	[31]
Dermaseptin S4 **Dermaseptin** (K4-S4-(1-28)) **Dermaseptin** (K4-S4-(1-16))	+4 +5 +4	*Phyllomedusa sauvagii* (Waxy monkey tree frog)		ALWMTLLKKVLKAAAKAALNAVLVGANA ALWKTLLKKVLKAAAKAALNAVLVGANA ALWKTLLKKVLKAAA	60 6 -	*C. albicans*	2.0 1.4 28	[32][33] [34][33] [33]
Tachykinin	+1	*Gallus gallus* (Red jungle fowl)	1N6T	HKTDSFVGLM	------	*C. albicans*	-	
Tritrpticin	+5	*Sus scrofa* (Wild boar)	2I1D	VRRFPWWWPFLRR	1000	*C. albicans*	-	[35]

AMPs as ionophores and its implication towards their antibacterial / antifungal activity

AMPs although being largely cationic have been shown to interact with metal cations of appropriate size[65,66] and possess the capability to import the metal ions inside bacterial or fungal cell creating an ionic gradient that leads to bloating of microbial cell and eventual rupture leading to its death (c.f. Fig. 1). This may be a possible mode of action for their antimicrobial activity as reported by our group. There is no experimental evidence for the antimicrobial mode of action of these peptides.

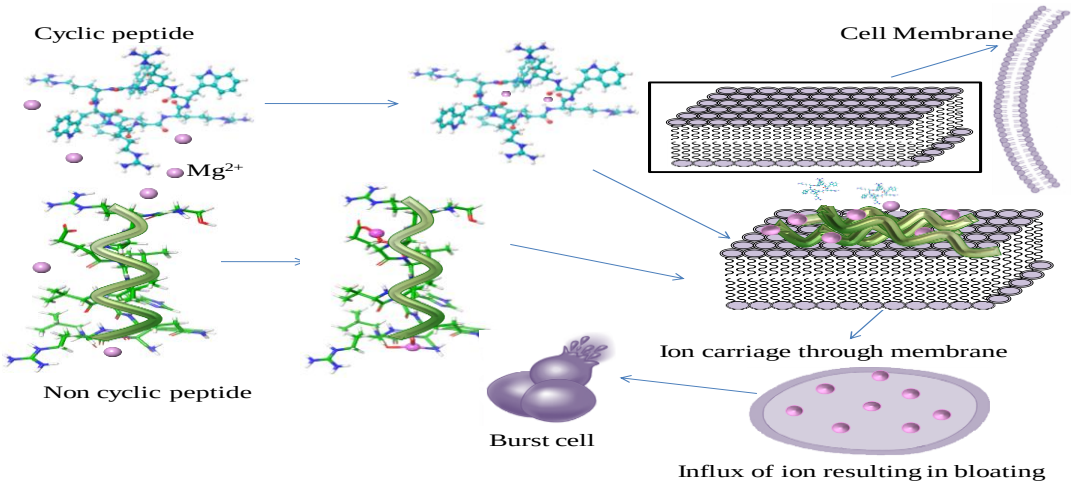


Fig 1: Ion carriage characteristics of AMPs.

Our research group has again performed ab initio intermolecular interaction calculations with complete geometry optimizations to understand the ionophore capability of some naturally occurring peptides and their peptidomimetic analogs (Figs. 2-4).

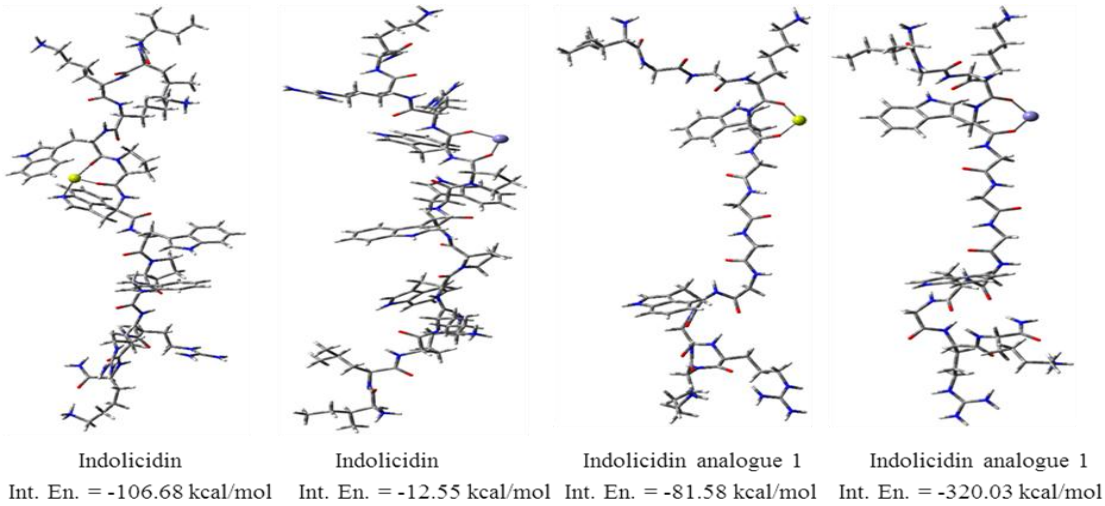


Fig.2 Indolicidin and Indolicidin analogue with Mg^{2+} and Fe^{2+} ions. (Mg^{2+} and Fe^{2+} ions are shown in yellow and purple respectively.)

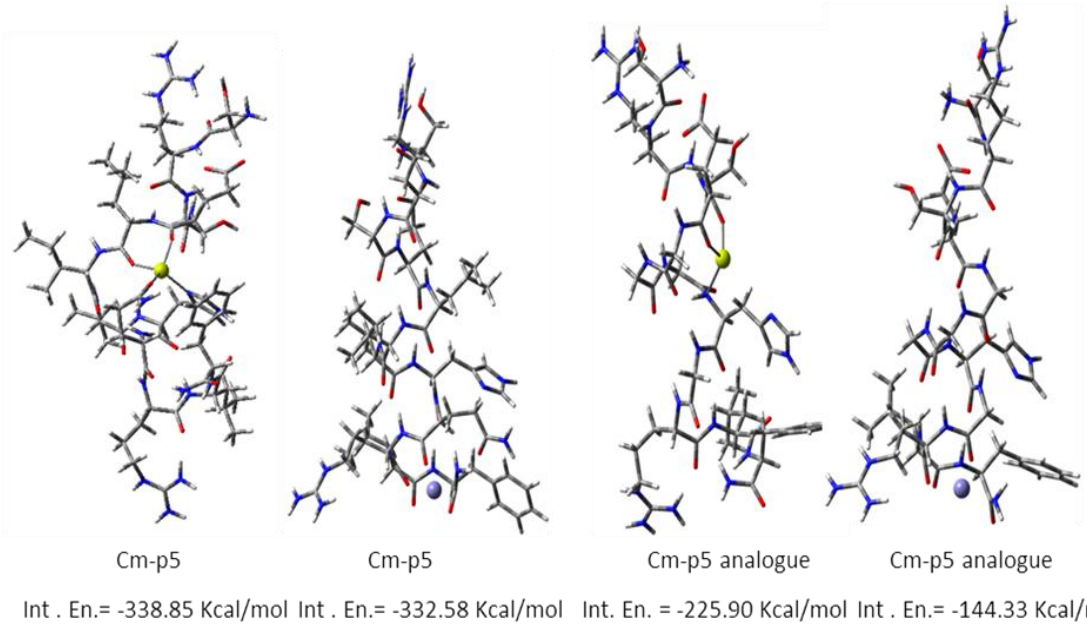


Fig.3 Cm-p5 and Cm-p5 analogue with Mg^{2+} and Fe^{2+} ions.
(Mg^{2+} and Fe^{2+} ions are shown in yellow and purple respectively.)

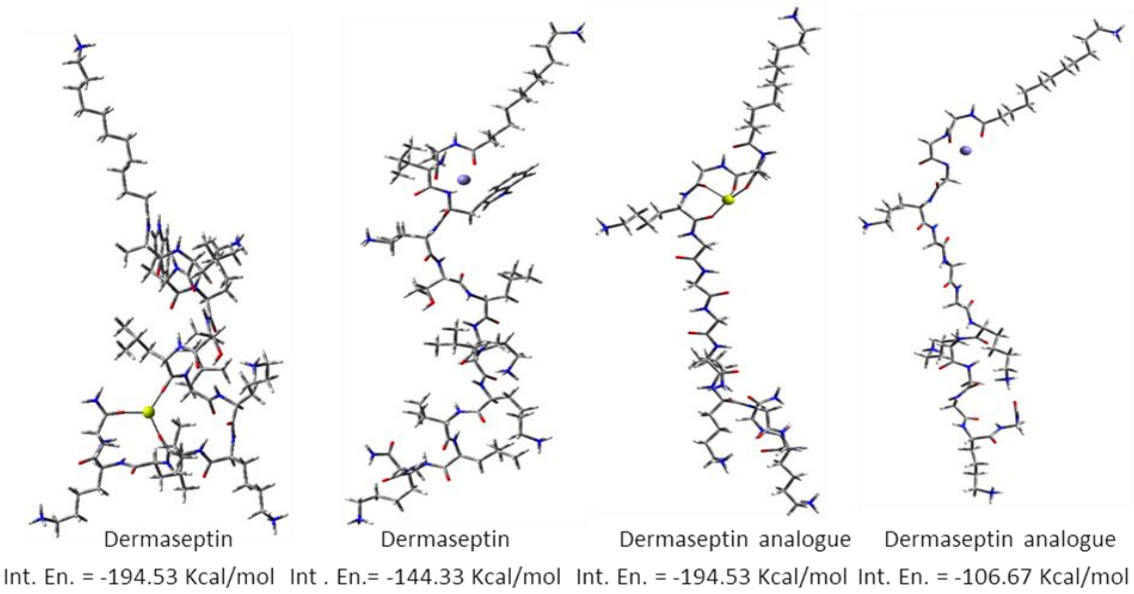


Fig.4 Dermaseptin and Dermaseptin analogue with Mg^{2+} and Fe^{2+} ions.
(Mg^{2+} and Fe^{2+} ions are shown in yellow and purple respectively.)

We have also computed the overall energetic gain in binding the ion after taking care of the loss, that is, the reorganization in peptide required to hold the ion efficiently (table 2).

Table 2: Antimicrobial Peptides and their ion carriage properties.

Antimicrobial Peptide Or Peptide Analogue	Charge	Sequence	Single Mg^{2+} ion carriage			Single Fe^{2+} ion carriage		
			Interaction energy (kcal/mol)	Energy required for peptide reorganization (kcal/mol)	Overall Stabilization (kcal/mol)	Interaction energy (kcal/mol)	Energy required for peptide reorganization (kcal/mol)	Overall Stabilization (kcal/mol)
Indolicidin variant (CP-11)	+6	ILKKWPWWPWRRK	-106.68	75.30	-31.37	-12.55	31.37	18.82
Cm-p5	+2	SRSELIVHQRLF	-338.85	94.13	-244.73	-332.58	119.23	-213.35
Dermaseptin (K4-S4-(1-13))	+5	ALWKTLLKKVLKA	-138.05	31.37	-106.68	-144.33	69.03	-75.30
Indolicidin analogue-1 (mutated indolicidin)	+4	IGGKWGGGGWGRK	-81.58	31.37	-50.20	-320.03	37.65	-282.38
Cm-p5 analogue-1 (mutated cm-p5)	+2	SRSEGGGHGRLF	-225.90	112.95	-112.95	-313.75	112.95	-200.80
Dermaseptin analogue-1 (mutated dermaseptin)	+5	GGGKGGGKKGGKG	-194.53	62.75	-131.78	-106.67	43.92	-62.75

Table 2 clearly indicates that the charge on peptide and its conformation both determine the overall affinity of peptide towards ion. The AMPs Fe^{2+} binding capacity in general may be responsible towards their bacterial 14-α demethylase enzyme inhibition capability as the enzyme requires Fe^{2+} ion for catalysis. This would then lead to weakened microbial cell wall and eventual lysis of microbial cell.

AMPs for metal toxicity removal therapy

Similar to the above mentioned Mg^{2+} and Fe^{2+} holding capacities of AMPs, heavy metal ions of similar size and charge may also be held by these peptides and removed from our body. Along these lines we have studied arginine and tryptophan rich cyclic peptides and also designed some peptidomimetic compounds as pharmaceutical leads for the development of drugs for Alzheimer's disease[67] and metal toxicity removal from human body.[66] Our ion binding results have been confirmed by difference absorption spectra (c.f. Fig. 5)

AMPs as anti-HIV agents

Many AMPs have shown anti-HIV activity along with antimicrobial activity as indicated by data collected in table 1. However, there have been druggability and pharmacokinetic issues in the past with peptide pharmaceuticals leading to their poor representation in drug market. Our belief is that mechanistic understanding can revive their status and bring these compounds to commercialization stage in a more meaningful way. As discussed in previous section, Mg^{2+} binding capability of AMPs could also contribute to their anti-HIV activity as many anti-HIV agents target these ions required for the catalytic activity of enzymes involved in replication of HIV virus. However, alternatively these peptides may interact with viral template or human DNA primer during reverse transcription process through interactions other than base pair-base pair or protein-protein interactions. Modern modeling and docking tools allow us to explore such mechanistic feasibilities. Our efforts in this direction and design of peptidomimetic compounds from there is discussed in following sections. It is expected that our efforts would help bring these mimetic compounds in clinical practices to curb microbial resistance issues and efficiently utilize their pharmaceutical potential for other diseases as well.

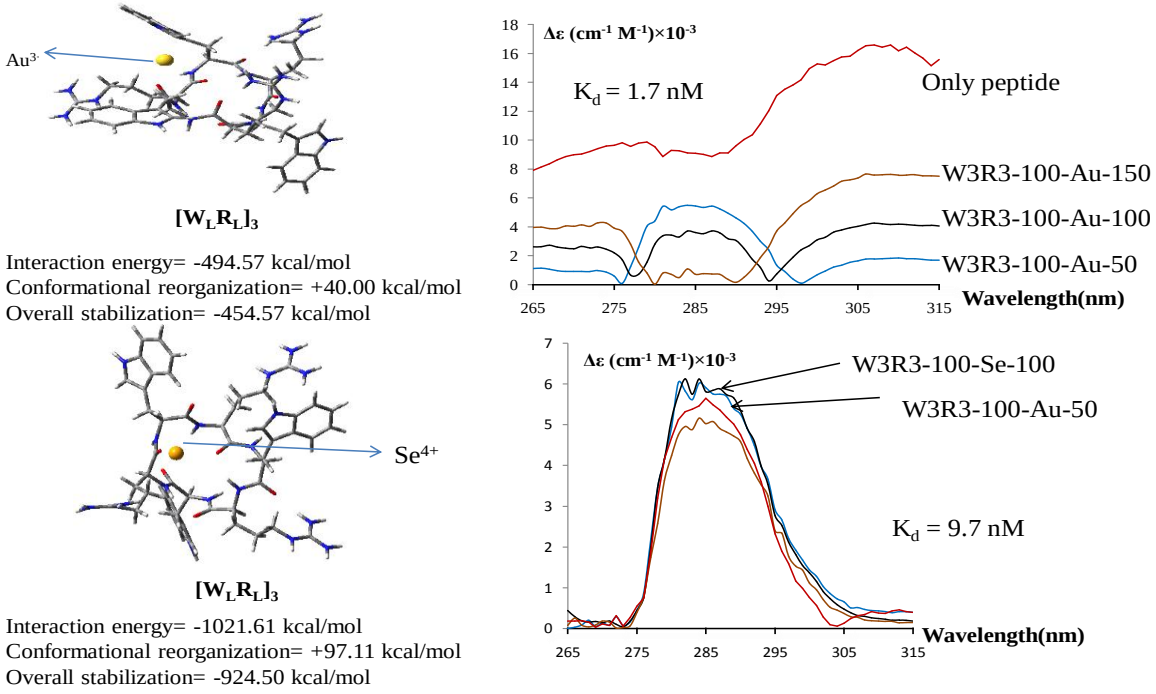
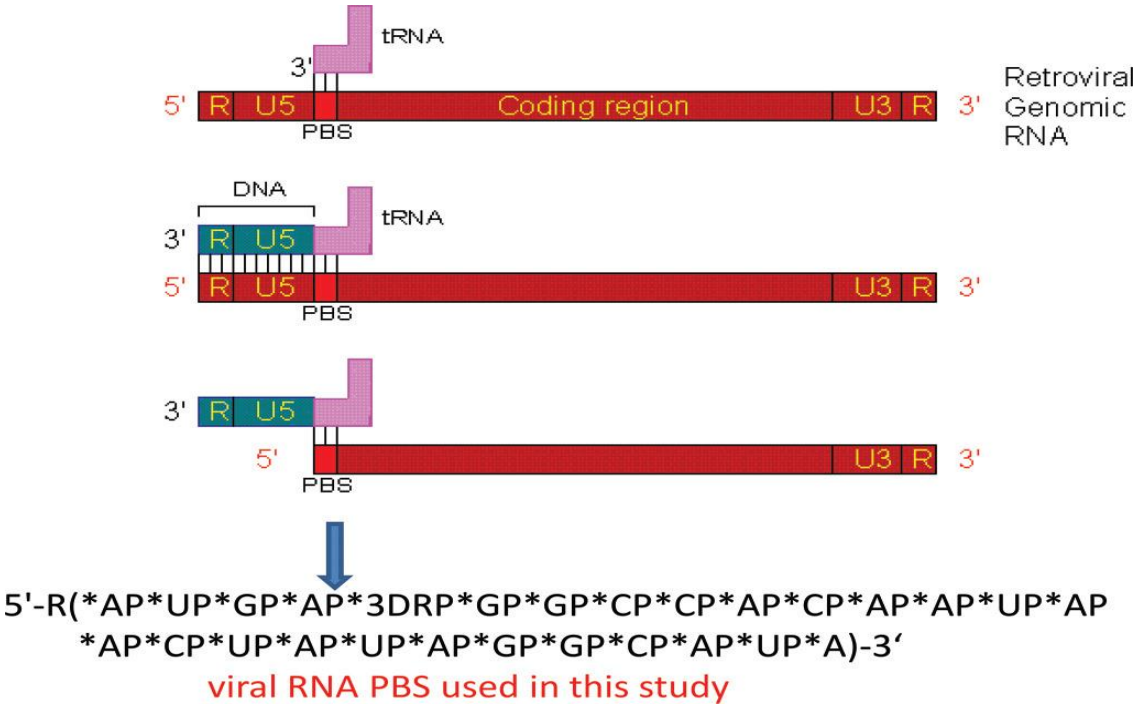


Fig 5: Non covalent complxation of Au^{3+} and Se^{4+} by $[W_LR_L]_3$ and corresponding changes with absorption spectra.

Inhibition of PBS of ss viral RNA template by AMPs

Human immunodeficiency virus type 1 (HIV-1) is a retrovirus and is the agent causing acquired immunodeficiency syndrome (AIDS). HIV-1 reverse transcriptase (HIV-1RT) is one of the several proteins encoded by viral genome and involved in its replication process. The RT enzyme of HIV-1 has two activities: a DNA polymerase that can copy either RNA or DNA templates and a Ribonuclease H (RNaseH) activity that hydrolyzes the RNA strand of an RNA–DNA hybrid. Most of the anti-HIV agents target different HIV-1 encoded enzymes, including polymerase activity of transcriptase, but none targets RNase activity. The mechanism of two Mg^{2+} ions is used by many nucleases and all polymerases. Hence, the drugs targeting Mg^{2+} ions show a lack of specificity. Compared with cellular RNase H1, viral RNase H prefers a longer template, i.e., >18 base pairs, and it fails to cleave the polypurine track (PPT) sequence in the viral genome, which can then prime the second DNA-strand synthesis. The HIV PBS is a structured RNA element in the genome of retrovirus to which tRNA binds to initiate reverse transcription. This 18-mer nucleotide piece follows the U5 region of the 5' long terminal repeat (LTR) of the retrovirus (Scheme 1). This work is focused at understanding the anti-HIV activity of some peptides used as drugs at the

molecular level through RT substrate inhibition as opposed to enzyme inhibition studies. We have considered the inhibition of the PBS of the ss viral RNA template, which primes the reverse transcription process. Modeled structures of chosen antimicrobial peptides were taken from Protein Data Bank and prepared for docking studies by adding hydrogen atoms and charges to atoms. The anti-HIV/anti-viral activity along with the antifungal activity of these peptides has been observed experimentally through cellular assays[33,68,70] but, the mechanistic aspects still remain ambiguous at the molecular level. The PBS of the ss viral RNA was taken from pdb file 4B3O corresponding to HIV-1RT ternary complex. The phosphate backbone of viral PBS at the point of cleavage by RNase is discontinuous. However, the entire PBS piece is intact, and the conformation is maintained as it is in its active position. This is the latest pdb containing RNA–DNA hybrid and contains the viral RNA PBS in catalytic conformation. If the template undergoes interactions with a peptide molecule, it may not be available for base pairing with the primer. This may then lead to an obstruction of polymerase activity.



Scheme 1: Early steps in the reverse transcription process along with the sequence of the viral RNA primer binding site used in this study. 3DR, 3-deoxyribose.

With the perception of such a mode of action, the prepared peptides were docked one by one to the viral template, and the interactions were analyzed in detail. Although a number of AMPs were studied, here we discuss our docking results and detailed, accurate interaction energy calculations on three peptides which have been used to design pharmaceutical leads. The dual docking protocol, standard followed by extra precision as mentioned in methodology section has been followed. Top few best poses were subjected to ab initio interaction energy calculations and also MMGBSA binding energy analysis.

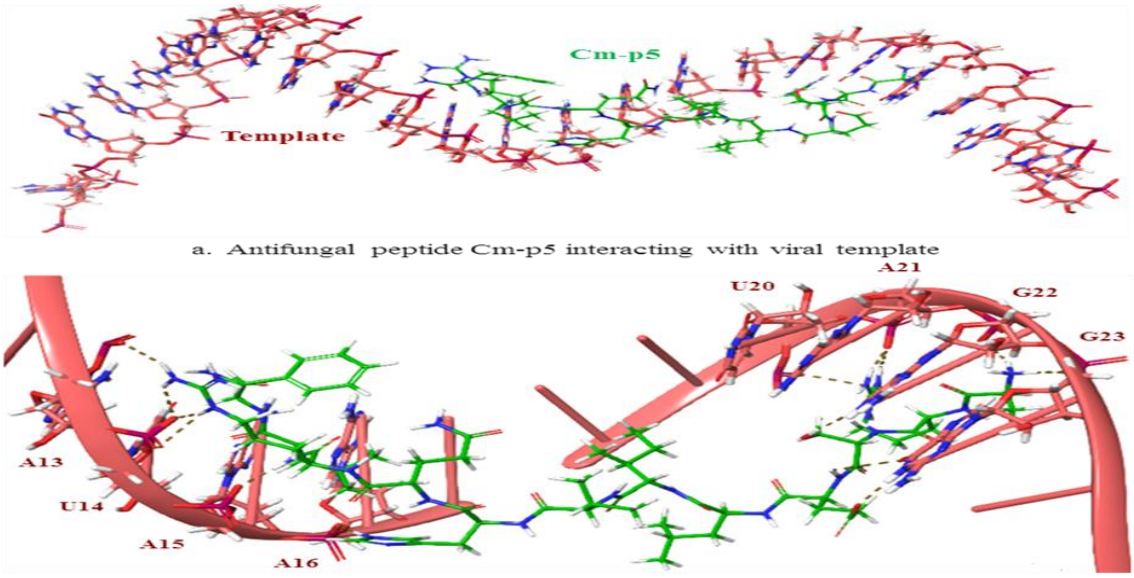
Figure 6 shows docking results for peptide Cm-p5. The peptide can be seen blocking the critical bend region of the viral template essential to reach out to the nuclease active site of the enzyme catalyzing the replication process. Close contacts within 4 Å are shown in the depicted pose. Near the free 5' end of the template and close to the polymerase active site, the template contains an abasic deoxyribose substitution marking the end of overhang portion. At the kink near the nuclease active site, the peptide can be seen interacting with the phosphate backbone and bases between A13 to G23. The ab initio interaction energy given in Fig. 6 estimates a strong electrostatic interaction between the AMP and the viral template. At this point, it is important to mention that DNA polymerases require a primer base-paired to the template for activity and in such a case when a peptide interacts with the template, the template becomes unavailable for reverse transcription process retarding and then eventually stopping the viral replication.

Docking results for dermaseptin are shown in Fig. 7. Compared with Cm-p5, this peptide is not only more cationic, but also its ab initio interaction energy is much more attractive. In other words, a tighter binding complex, This peptide also obstructs the middle kink region of the template in the vicinity of nuclease hydrolysis activity.

The interactions of indolicidin are shown in Fig. 8. This peptide shows good contribution from electrostatic interactions towards glide energy. The conformation of indolicidin is most appropriate for interactions with the template along with its cationic residues, which result in maximum interactions with the viral template, making it the most suitable lead peptide. It is observed that mostly cationic and aromatic residues are involved in binding interactions. The ab initio interaction energies clearly indicate a strong electrostatic contribution towards binding energy. Highly cationic peptides show a stronger interaction with the viral template. The effect of solvent on binding energies has also been studied by placing implicit solvent around the complexes and evaluating MMGBSA binding energies. To reaffirm that poses

generated correspond to stable complexes we have evaluated ab initio interaction energies as well. MMGBSA binding energy contributions indicate that contributions from coulombic attractions and van der Waals interactions are the most important.

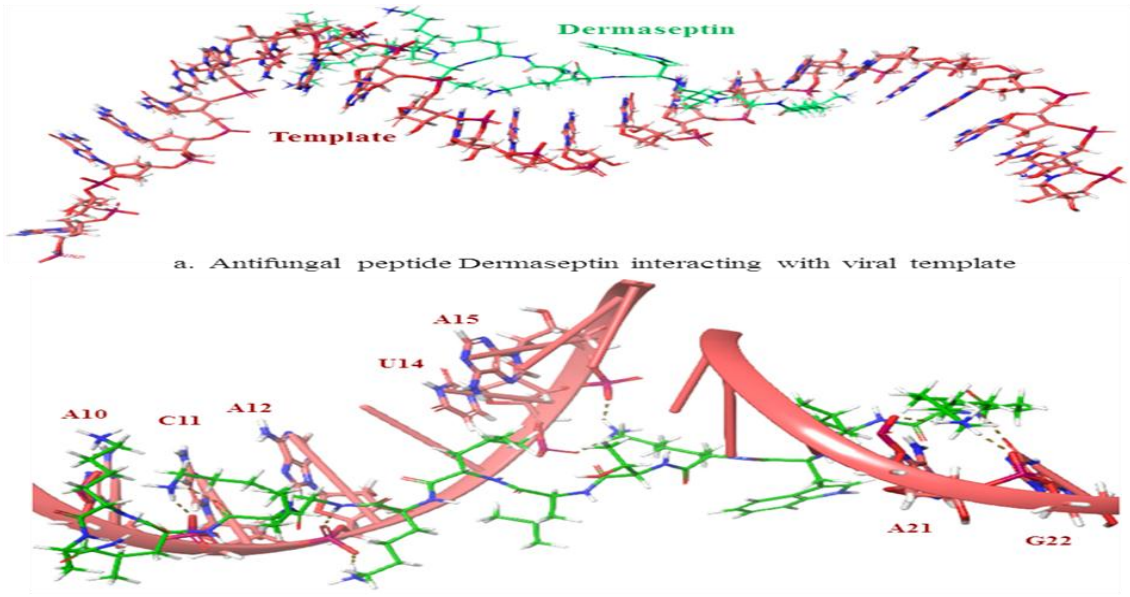
To summarize, the docking results indicate that cationic residues in the antimicrobial peptide enhance the binding interactions as long as the sequence results in appropriate conformation to interact with the template.



a. Antifungal peptide Cm-p5 interacting with viral template

b. A close up view of interactions between peptide and template
Interaction Energy = -1255.02 kcal/mol

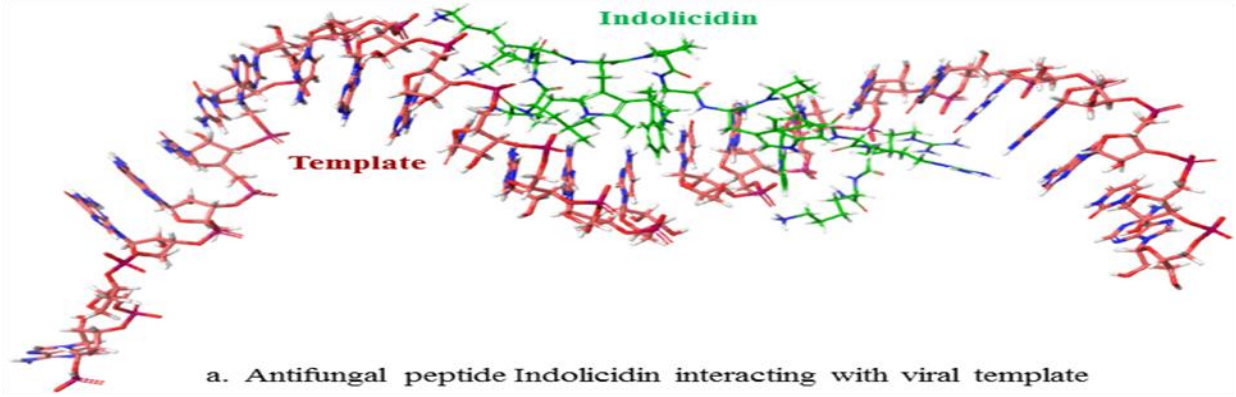
Fig. 6 Antifungal peptide Cm-p5 inhibiting viral template



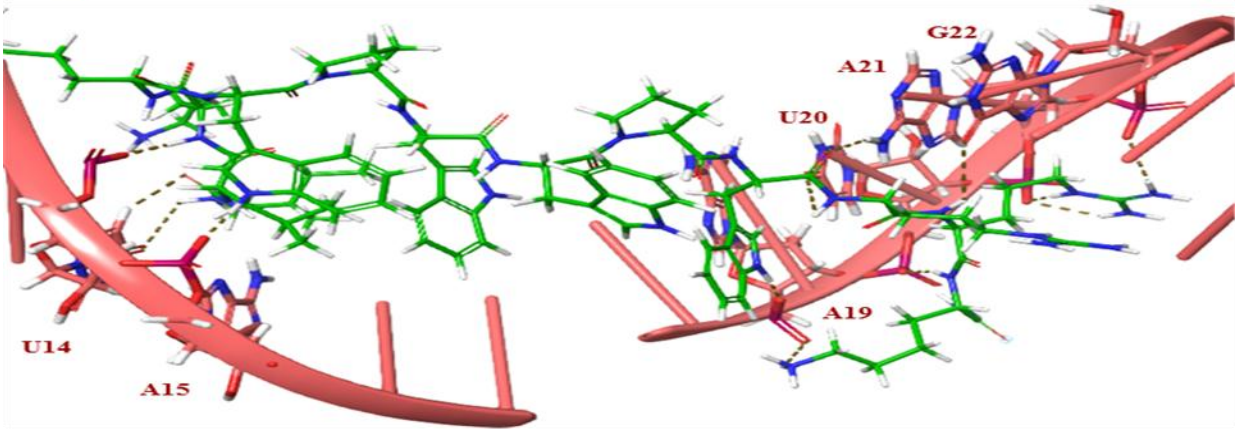
a. Antifungal peptide Dermaseptin interacting with viral template

b. A close up view of interactions between peptide and template
Interaction Energy = -2572.791 kcal/mol

Fig. 7 Antifungal peptide Dermaseptin inhibiting viral template



a. Antifungal peptide Indolicidin interacting with viral template



b. A close up view of interactions between peptide and template
Interaction Energy = -2867.72 kcal/mol

Fig. 8 Antifungal peptide Indolicidin inhibiting viral template

Design of peptidomimetic pharmaceutical leads

After understanding the anti-HIV activity of these peptides, we computed their ADME properties to understand why these compounds have not succeeded clinically and what could possibly be the strategy to increase their druggability. Calculated ADME properties are given in table 3, which indicate that we need to drastically cut down on molecular weight and the number of hydrogen bond donors and acceptors to overcome adverse pharmacokinetic issues of these peptides. Our systematic efforts to enhance druggability features are discussed in following section and depicted systematically in scheme 2. As all the three studied AMPs showed good anti-HIV features and we are interested in utilizing 'other' pharmacological properties of these peptides along with their antimicrobial activity, it seemed logical to start designing from all the three peptides.

The first step was molecular weight reduction, which was accomplished by assessing and then mutating the non interacting residues to glycine. The non interacting residues were identified after detailed contact analysis (c.f. table 4). This led to significant decrease in molecular weight.

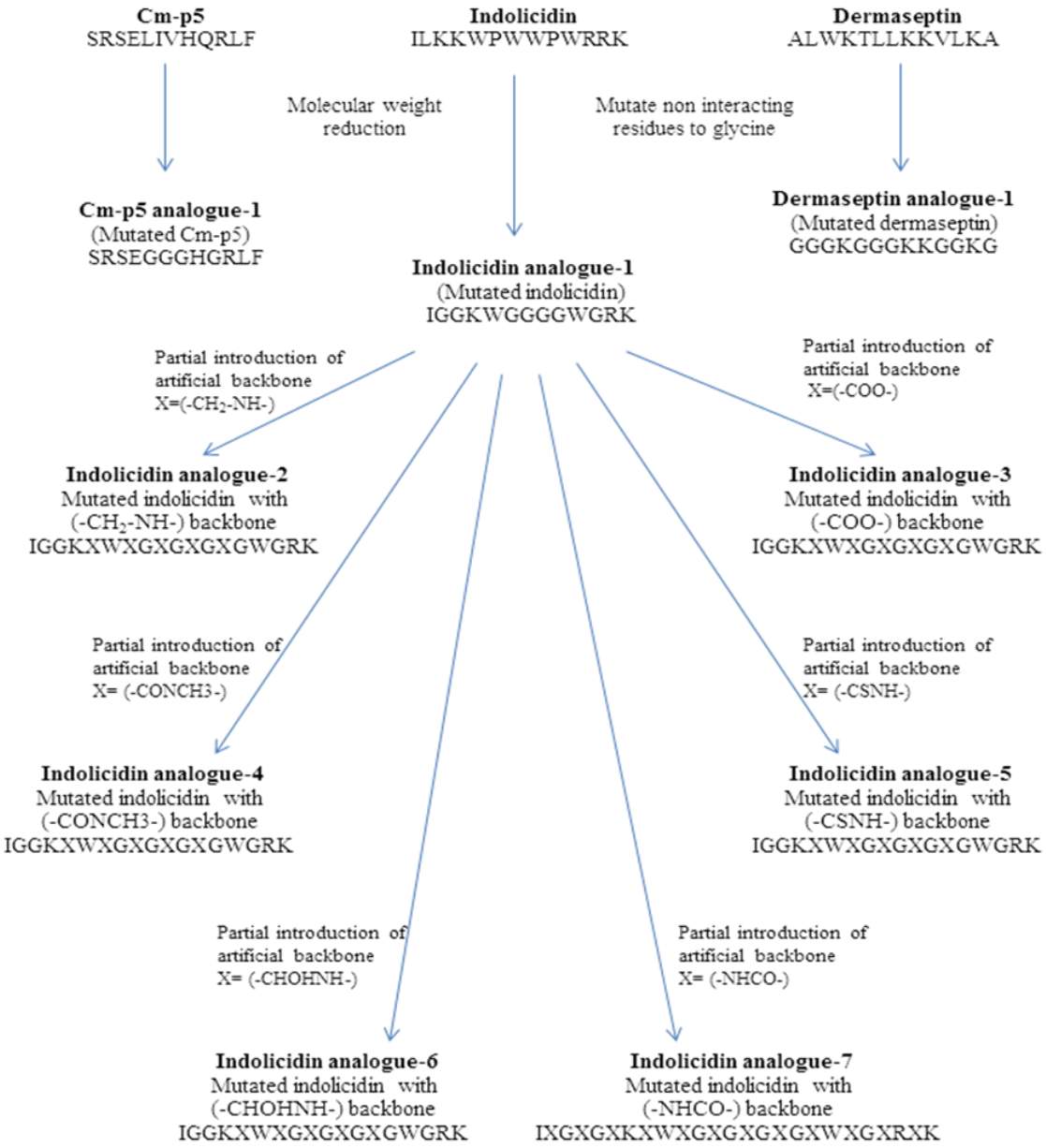
The mutated analogues of the three peptides that is, indolicidin analogue 1, dermaseptin analogue 1 and Cm-p5 analogue 1 were prepared utilizing protein preparation wizard and docked into the HIV viral template to study their interactions. Same methodology was followed as before.

All the three analogues gave significant interactions with the template. Since indolicidin analogue was reasonably cationic and of appropriate conformation it was carried further for enhancement of druggability features. The ADME properties (c.f. table 3) are still not in the desired range but better than the natural peptide.

Table 3. ADME properties* of antifungal peptides and designed peptidomimetic compounds.

Antifungal Peptides	Molecular weight	Solvent accessible surface area Unit Å^2	Number of hydrogen bond donor	Number of hydrogen bond acceptor	Globularity	Hydrophobicity	% Oral absorption	Number of violations of Lipinski's rule
Indolicidin variant (CP-11)	1879.327	2507.674	24.500	34.000	0.596	-1.137	0	3
Cm-p5	1483.732	2116.350	18.750	34.650	0.611	-7.113	0	3
Dermaseptin (K4-S4-(1-13))	1708.288	2562.475	16.250	30.950	0.576	1.589	0	3
Indolicidin analogue-1 (mutated indolicidin)	1314.512	1937.908	17.000	28.500	0.618	-5.941	0	3
Indolicidin analogue-2 (mutated indolicidin with (-CH_2NH-)backbone)	1244.594	2119.563	21.500	28.000	0.576	-5.391	0	3
Indolicidin analogue-3 (mutated indolicidin with (-COO-) backbone)	1319.436	2018.986	15.750	29.750	0.598	-3.338	0	3
Indolicidin analogue-4 (mutated indolicidin with (-CONCH3-) backbone)	1384.646	2098.927	15.750	34.750	0.597	-6.207	0	3
Indolicidin analogue-5 (mutated indolicidin with (-CSNH-) backbone)	1394.815	1944.919	20.750	29.750	0.624	-1.903	0	3
Indolicidin analogue-6 (mutated indolicidin with (-CHOHNH-) backbone)	1324.591	1815.304	26.500	36.500	0.648	-10.867	0	3
Indolicidin analogue-7 (mutated indolicidin with entire(-NHCO-) backbone)	1314.512	1768.635	15.500	27.000	0.661	-4.837	0	3
Cm-p5 analogue-1 (mutated cm-p5)	1258.359	1932.865	16.750	32.150	0.604	-9.778	0	3
Dermaseptin analogue-1 (mutated dermaseptin)	1240.511	1846.763	15	30	0.642	-8.588	0	3

***Permissible range:**
Molecular Weight 130 - 725, Total SASA 300 - 1000 Å^2, Number of hydrogen bond donors 0 - 6
Number of hydrogen bond acceptors 2 - 20, Globularity 0.75 - 0.95, Hydrophobicity log Po/w -2 – 6.5



Scheme 2 Design strategy for a peptidomimetic inhibitor of HIV-1 RT.

Table 4: Contact analysis for docked antifungal peptides.

Antifungal peptide	Peptide ligand sequence and interacting residue (underlined)	Template* interacting region
Indolicidin variant (CP-11)	ILKKWPWWPWRRK	Backbone U14
	ILKKWPWWPWRRK	Backbone U14
	ILKKWPWWPWRRK	Backbone A15
	ILKKWPWWPWRRK	Backbone A19,U20(base),A21
	ILKKWPWWPWRRK	Backbone and base G21, G22
	ILKKWPWWPWRRK	Backbone A19
Cm-p5	SRSELIVHQRLF	Backbone and base,G22,G23
	SRSELIVHQRLF	Backbone,A21,U20
	SRSELIVHQRLF	Backbone,G22, G23
	SRSELIVHQRLF	Backbone,G23
	SRSELIVHQRLF	Backbone,A16
	SRSELIVHQRLF	Backbone,A13, A14(base)
	SRSELIVHQRLF	Backbone,A15(base)
	SRSELIVHQRLF	Backbone, U14,A15(base)
Dermaseptin (K4-S4-(1-13))	XALWKTLLKKVLKA	Backbone A21, G22(base),
	XALWKTLLKKVLKA	Backbone U14(base), A15
	XALWKTLLKKVLKA	Backbone A12
	XALWKTLLKKVLKA	Backbone A10,C11,A12
	XALWKTLLKKVLKA	Backbone A10

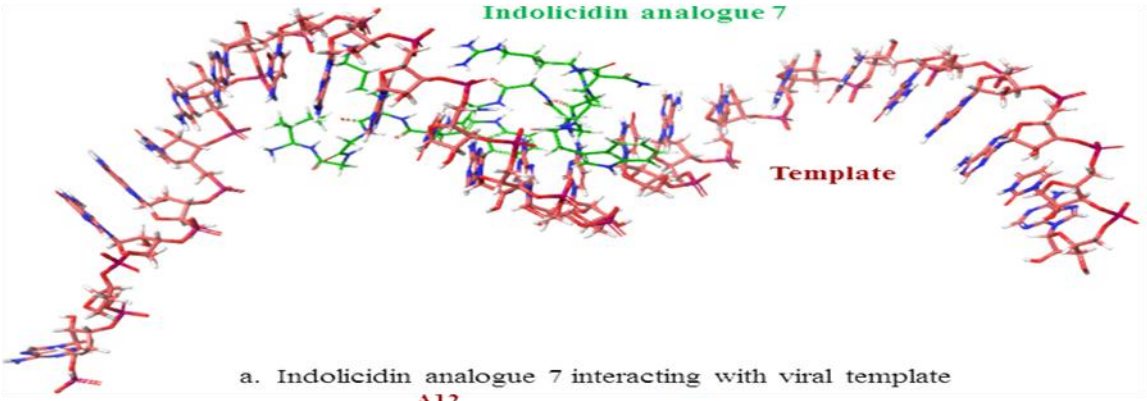
*Template sequence
5'(pApUpGpA4p3DR5pG6pGpCpCpApCpA12pA13pUpApApCpU18pA19pUpApGpGpC24p A25pUpAp) 3'

The next design step was to overcome proteolytic issues by partial introduction of artificial backbone, as well as concurrent reduction in molecular weight if possible.

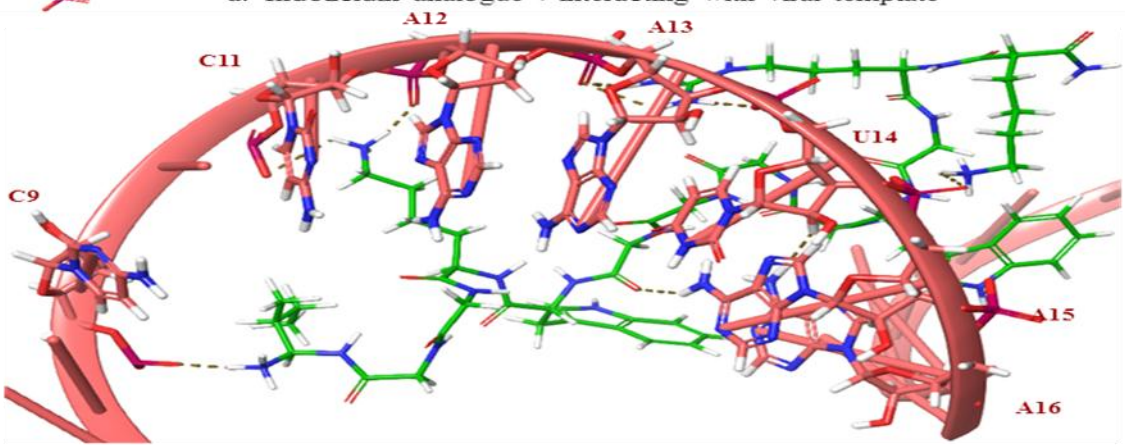
The peptide backbone of indolicidin analogue 1 was partially replaced by different backbones in the mutated part one by one namely $–CH_2NH–$ backbone, -COO- backbone, $-CONCH_3-$ backbone, -CSNH- backbone, -CHOHNH- backbone. ADME properties of these indolicidin analogues 2 to 6 are shown in table 3 which indicates minor improvement in pharmacokinetic aspects of these analogues at the expense of increase in molecular weight in general. Therefore, it was intuitively decided to try –NHCO- artificial backbone throughout which is the closest to peptide backbone, does not increase molecular weight and yet helps overcome proteolytic issues. The docking results for this analogue are shown in fig.9. The interaction energy of this analogue is comparable to natural indolicidin and dermaseptin. It also blocks the crucial kink region of viral template and thus appears to be the best as a starting point.

This indolicidin analogue 7 is therefore recommended as the major pharmaceutical lead for the development of drugs for HIV and internal fungal infections which are both intertwined deadly diseases. The intertwining is a result of decreased immunity in HIV patients which requires administration of long term, low dose safe antifungal to avoid the risk of systemic internal fungal infections that are almost impossible to deal with existing antibacterial/ antifungal agents.

This study indicates that with the help of modern molecular modeling and docking tools it is possible to design and virtually screen pharmaceutical leads with moderate ADME characteristics. Such studies shall have significant impact on pharma industry and can help them save time and money in the discovery of beneficial drugs for society. The pharmaceutical lead thus designed may not be ideally the best but, it is encouraging to see that there is wide scope for development of good pharmaceutical leads based on molecular modeling and docking studies.



a. Indolicidin analogue 7 interacting with viral template



b. A close up view of interactions between peptidomimetic compound and template
Interaction Energy = -2045.6826 kcal/mol

Fig. 9 Indolicidin analogue 7 inhibiting viral template.

CONCLUDING REMARKS

In this study mechanistic aspects of some AMPs have been studied through modern molecular modeling and docking tools. Based on the mechanistic understanding an attempt has been made to systematically enhance druggability features for the better utilization of these compounds in medicine. Molecular modeling and docking tools have been extensively used to perform mutations and introduce artificial backbone to enhance ADME properties and pharmacokinetics of designed compounds. It is not easy to mutate selected residues in AMP and especially backbone alterations are almost impossible without modeling and docking tools of Maestro utilized herein. Hence, these modern computational tools are an indispensable part of pharmaceutical research and help save a lot of pharma company revenue. The peptidomimetic compounds are expected to overcome microbial resistance issues and proteolytic complications as well. An analogue of a naturally occurring AMP has been suggested as lead compound for the development of safe antifungal with anti-HIV activity to deal with intertwined fatal diseases HIV and internal fungal infection. This study opens the path for experimental chemists working in this field to enhance druggability features of peptidomimetic compounds in pursuit of non toxic, robust pharmaceuticals.

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