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A mechanistic study of anti HIV activities of antifungal peptides

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Abstract

HIV patients are constantly at risk of developing internal fungal infection and are thus regularly prescribed antifungal medications. Several classes of antifungal agents have thus been developed to combat ever increasing cases of resistant strains of fungi. Azoles despite being the most popular clinical choice; are not devoid of side effects. Many antimicrobial peptides have also been tested in search of safe, non toxic antifungals but, none succeeded as commercial alternatives. Recent research attempts show continued interest in these compounds and the complexities associated. Some experimental observations indicate involvement of these antimicrobial peptides in enhancing the efficacy of anti HIV agents. We present here an intertwined approach to deal with two fatal diseases, internal fungal infection and HIV infection. Several naturally occurring antimicrobial peptides have been studied for their possible interaction with the viral RNA primer binding site (template) through interactions other than base-pair–base-pair type. Peptides have been prepared and docked into viral template utilizing extra precision, flexible ligand docking. Implicit solvent was added around the complex and MMGBSA interaction energies were computed. Druggability aspects were explored by calculating ADME related properties. A peptidomimetic compound has been strategically designed to introduce some druggability features in the peptide maintaining its viral template inhibition capability. Designed peptidomimetic lead compound may help in obtaining non toxic anti HIV agents in future. This is the first study to suggest a plausible explanation for the anti HIV activity of antifungal peptides at the molecular level and corroborate experimental findings of synergistic effects of these peptides on anti HIV agents.

Keywords: antifungal peptide, anti HIV agent, viral template inhibition, primer binding site, indolicidin

Introduction

Antifungal agents are primarily used for the treatment and prevention of human fungal diseases like candidiasis, ring worm etcetra¹. Antifungal agents are used topically, orally² as well as administered intravenously to treat severe systemic infections like cryptococcal meningitis³. Topical antifungal preparations usually do not cause any side effects. Oral antifungal drugs cause few common side effects like nausea, diarrhoea and headache⁴. However, the antifungals injected intravenously for severe internal infections, for example, Amphotericin B cause serious problems and are administered only when the need for treatment outweighs their risk⁵. The need for safe antifungals has increased multifold with rising number of AIDS patients and everyday increase in resistant strains of fungi. AIDS patients suffer from poor immune system and are thus, at constant risk of developing internal fungal infection. Such patients are prescribed regular antifungal medications⁶. Long term antifungal medications obviously require safer drugs as compared to commonly prescribed azole compounds. Many antimicrobial peptides (AMPs) have been isolated, tested, synthesized in search of safe, non toxic antifungals⁷⁻¹¹. Some AMPs were found to be cytotoxic against mammalian cells⁷ but few did not cause hemolysis of human erythrocytes and were considered safe for human use. While evaluating the antifungal property of some AMPs, they were observed to enhance the efficacy of anti HIV agents and were also suspected to be involved in inhibiting enzymes required for the replication of virus¹². HIV virus inhibitory activities of some naturally occurring peptides have been collected in table 1¹³⁻¹⁷.

The aim of this study is to explore the possibility of some AMPs inhibiting the viral transcription process by interacting with the viral RNA template, through interactions other than base pair-base pair type. Some natural AMPs classified as antifungal agents have been chosen for the study to achieve triple benefit of antifungal, anti HIV activities and cell permeability properties. This study

will provide a molecular level rationale for the observed anti HIV activity of some peptides. After understanding the mechanistic aspects, the study also aims to propose modifications in peptide structures to enhance their druggability. Peptide drugs in particular suffer from proteolytic problems and slow absorption due to their high molecular weights. Peptidomimetic compounds may thus emerge as better alternatives to treat severe systemic fungal infections and HIV infections simultaneously.

Methodology

A combination of molecular modeling, docking and MM/GBSA binding energy calculations have been performed to study inhibition of HIV viral replication process by small antifungal 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. Following sections give a brief account of methods used:

Peptide and template preparation

Modeled structures based on solution NMR data have been reported for several AMPs and are available in the Brookhaven protein databank. Sequences of five naturally occurring small antifungal peptides chosen for the study are given in table 2¹⁸⁻²². 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 software²³. The primer binding site (PBS) of HIV viral single-stranded (ss) RNA was taken from pdb Id 4B3O²⁴ corresponding to 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 Schrodinger software.

Docking studies

To study the interaction of chosen AMPs with the PBS of viral template and to judge its capability to block PBS, the peptide was now docked into the template choosing entire PBS as target. Grid was placed at the center of the template. Standard precision (SP) flexible ligand docking with post docking minimization was performed utilizing Glide module²⁵ of Schrodinger software. Best poses from SP docking were subjected to extra precision (XP) 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 post docking minimization out of which best 10 were filtered. For the studied peptides a maximum of three poses were generated in each case after post docking minimization. Since docking has been performed to a stretch of ssRNA as opposed to the binding pocket of any rigid protein it is prone to 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 set²⁶ and subsequently Combined Molecular mechanics Generalized Born Surface area (MMGBSA) binding energy evaluation with implicit solvent around the complex.

Quantum mechanical ab initio (gas phase) interaction energy and MM/GBSA (after implicit solvation) binding energy evaluations

Ab initio molecular orbital calculations have been performed at the Hartree Fock (HF)/ 6-31G²⁶ level utilizing Gaussian '09 software²⁷. Interaction energy between peptide and template has been calculated by supermolecule approach without basis set superposition error (BSSE) as follows:

$$\text{Interaction energy} = E_{\text{complex}} - (E_{\text{peptide}} + E_{\text{template}})$$

MMGBSA calculations have been performed using Prime module of Schrodinger software²⁸ with Maestro interface. MMGBSA approach uses molecular mechanics OPLS 2005 force field coupled with generalized Born surface area continuum solvent model for the prediction of solvation energies of complex and the two fragments²⁹. Binding energies are then evaluated as follows:

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

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.

Calculation of druggability parameters and design of peptidomimetic lead compound

ADMET properties have been calculated utilizing QikProp module³⁰. An AMP with the best interaction energy was then chosen and modified strategically to enhance druggability features to achieve a lead compound for the development of safer, non toxic antifungal agent with anti HIV activity in future.

Results and Discussion

Human immunodeficiency virus type 1 (HIV-1) is a retrovirus and is the agent causing Auto immunodeficiency syndrome (AIDS). HIV 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 (RNase H) activity which hydrolyzes the RNA strand of an RNA/DNA hybrid (fig. 1). Most of the anti HIV agents target different HIV-1 encoded enzymes including polymerase activity of transcriptase but none target RNase activity. Two Mg^{2+} -ion mechanism is used by many nucleases and all polymerases. Hence, the drugs targeting Mg^{2+} ions show lack of specificity. Viral RNase H prefers longer template, that is, > 18 base pairs as compared to cellular RNase H1 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 primer binding site (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 I). This work is focused at understanding the anti HIV activity of some peptide drugs at the molecular level through RT substrate inhibition as opposed to enzyme inhibition studies. We have considered the inhibition of PBS of ss viral RNA template priming the reverse transcription process.

Modeled structures of chosen antifungal peptides were taken from protein databank and prepared for docking studies by adding hydrogen atoms and charges to atoms. The twisted conformations of these peptides used in docking studies are shown in fig. 2. The anti HIV/ anti viral activity alongwith the antifungal activity of these peptides has been observed experimentally through cellular assays³¹⁻³⁴ but the mechanistic aspects still remain ambiguous at the molecular level. The PBS of 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 maintained as in its active position.

This is the latest pdb containing RNA/DNA hybrid. It contains the viral RNA PBS in catalytic conformation.

Now the prepared peptides were docked one by one to the viral template and the interactions were analyzed in detail. Fig. 3 shows docking results for Cm-p5. 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 4Å are shown in the depicted pose. Near the free 5' end of the template close to the polymerase active site the template contains an abasic deoxy ribose 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. At this point it is important to mention that DNA polymerases require a primer base-paired to template for activity. 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 obstruction of polymerase activity. XP Docking score, glide energy and its main components are given in table 3. Detailed residue wise contact analysis is given in table 4.

Docking results for dermaseptin are shown in fig. 4. This peptide is more cationic as compared to Cm-p5. Its ab initio interaction energy is much more attractive as compared to Cm-p5. This peptide also obstructs the middle kink region of template in the vicinity of nuclease hydrolysis activity. Table 4 clearly indicates the cationic lysine residues of dermaseptin involved in hydrogen bonding with neighbouring bases on the template. Fig. 5 shows tachykinin anchored to template mainly through hydrogen bonding as it contains lesser number of cationic residues and therefore less electrostatic interaction. However, tachykinin can also inhibit the replication of virus as its conformation is appropriate to form non bonded interactions for anchorage. The close-up view in fig. 5b clearly shows the non bonded close contacts.

The interactions of tritrpticin and indolicidin, two highly cationic peptides are shown in figs. 6 and 7. Both the peptides show good contribution from electrostatic interactions towards glide energy. The three tryptophan residues in tritrpticin do not seem to contribute much towards interactions with the template. The indolicidin conformation on the other hand is most appropriate for interactions with template along with its cationic residues which result in maximum interactions with the viral template making it the most suitable lead peptide. Detailed contact analysis for all the poses is given in table 4. It is evident from the table that mostly cationic and aromatic residues are involved in binding interactions. The ab initio interaction energies clearly indicate strong electrostatic contribution towards binding energy. Highly cationic peptides show stronger interaction with viral template.

The effect of solvent on binding energies has been studied by placing implicit solvent around the complexes and evaluating MM/GBSA binding energies which are compiled in table 3. Binding energy contributions indicate coulombic and van der Waals contributions to be the most important. Cationic residues in the antifungal peptide enhance the coulombic contribution as long as the sequence results in appropriate conformation. Dermaseptin despite being highly cationic resulted in poorer binding energy as compared to tritrpticin.

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 5 which indicate that we need to drastically cut down on molecular weight, number of hydrogen bond donors and acceptors. Our systematic efforts to enhance druggability features are described in following section and shown schematically in Scheme II.

Since indolicidin gave the best binding energy, it seemed logical to start designing from there. The first step was molecular weight reduction which was accomplished by mutating the non-interacting residues to glycine. Interacting residues were assessed from table 4. This led to significant decrease in molecular weight.

The mutated peptide labeled as 'Designed compound 1' was then prepared utilizing protein preparation wizard and docked into template to study its interaction. Docking results are shown in fig. 8. Designed compound 1 shows significant interaction with template before and after solvation though the positive charge has been reduced. The ADME properties (c.f. table 5) are still not in the desired range but better than natural peptide. The next design step was to overcome proteolytic issues by partial introduction of artificial backbone with concurrent reduction in molecular weight as well. The peptide backbone of designed compound 1 was replaced with $-\text{CH}_2\text{NH}-$ in the non interacting part resulting in Designed compound 2. Designed compound 2 was then prepared and docked. Docking results are shown in fig. 9. Designed compound 2 also shows interactions similar to designed compound 1. The coulombic contribution to binding energy has decreased due to less interacting $-\text{CH}_2\text{NH}-$ backbone. Table 3 also gives an analysis of binding energy components for DNA primer-viral template base pair-base pair interactions which are perfectly complementary to each other. The analysis indicates highly repulsive coulombic contribution due to the negatively charged phosphate backbones of both brought together. The electrostatic solvation contribution indicates favorable solvation of base pairs as opposed to individual strands which results in overall highly attractive binding energy. If the drug is to act competitively it must bind to viral template with binding energy comparable to that of primer-template interactions. In an attempt to enhance the binding energy another designed compound 3 with slightly more polar ($-\text{COO}-$) artificial backbone was considered, prepared and docked; the

results of which are depicted in fig. 10. Results of binding energy before and after solvation were similar to designed compound 2.

In brief, the designed peptidomimetic compounds although possess better druggability, they show binding energy weaker than natural peptide backbone. Also, when competing with primer for template show poor competitive inhibition. Compared to natural peptides which do show anti HIV activity³¹⁻³⁴, designed compound 1 seems to be a good compromise between druggability and anti HIV activity. The encouraging aspect is that there is wide scope for development of a good, competitive peptidomimetic inhibitor of HIV-1RT. The mechanistic elucidation from this study will certainly help in this direction.

Concluding Remarks

In this study some natural, small antifungal peptides have been studied for their anti-HIV activity. A possible mode of action for their viral inhibitory activity has been proposed. Efforts have been made to enhance druggability features of these peptides by mutating and introducing artificial backbone. Although the backbone alteration efforts were not so successful but this study is a preliminary step towards development of non toxic drugs for the combined treatment of two fatal diseases internal fungal infection and HIV infection.

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Figure Captions

Fig. 1 HIV-1RT ternary complex

Fig. 2 Conformations of selected antifungal peptides

(Sequences of peptides are shown in red color)

Fig. 3 Antifungal peptide Cm-p5 inhibiting viral template

Fig. 4 Antifungal peptide Dermaseptin inhibiting viral template

Fig. 5 Antifungal peptide Tachykinin inhibiting viral template

Fig. 6 Antifungal peptide Tritrpticin inhibiting viral template

Fig. 7 Antifungal peptide Indolicidin inhibiting viral template

Fig. 8 Designed Compound 1 inhibiting viral template

Fig. 9 Designed Compound 2 inhibiting viral template

Fig. 10 Designed Compound 3 inhibiting viral template

Fig. 1 HIV-1RT ternary complex

3DR= 3 deoxy ribose

Scheme I Early steps in the reverse transcription process alongwith the sequence of viral RNA primer binding site used in this study

Cm-p5**SRSELIVHQRLF****PDB ID: 2MP9** **Dermaseptin****ALWKTLKKVLKA****PDB ID: 2DCX** **Tachykinin****HKTDSFVGLM****PDB ID: 1N6T** **Tritrpticin****VRRFPWWPFLRR****PDB ID: 2I1D** **Indolicidin****ILKKWPWWPWRK****PDB ID: 1QXQ**

Fig. 2 Conformations of selected antifungal peptides
(Sequences of peptides are shown in red color)

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. 3 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 = -2572.79 kcal/mol

Fig. 4 Antifungal peptide Dermaseptin inhibiting viral template

a. Antifungal peptide Tachykinin interacting with viral template

b. A close up view of interactions between peptide and template
Interaction Energy = -822.04 kcal/mol

Fig. 5 Antifungal peptide Tachykinin inhibiting viral template

a. Antifungal peptide Tritrpticin interacting with viral template

b. A close up view of interactions between peptide and template
Interaction Energy = -2340.61 kcal/mol

Fig. 6 Antifungal peptide Tritrpticin 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. 7 Antifungal peptide Indolicidin inhibiting viral template

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

a. Designed Compound 1 interacting with viral template

b. A close up view of interactions between peptidomimetic compound 1 and template
Interaction Energy = -1970.38 kcal/mol

<https://mc.manuscriptcentral.com/cjc-pubs>
Fig. 8 Designed Compound 1 inhibiting viral template

a. Designed Compound 2 interacting with viral template

b. A close up view of interactions between peptidomimetic compound and template
Interaction Energy = -2026.86 kcal/mol

Fig. 9 Designed Compound 2 inhibiting viral template

a. Designed Compound 3 interacting with viral template

b. A close up view of interactions between peptidomimetic compound and template
Interaction Energy = -2033.13 kcal/mol

Fig. 10 Designed Compound 3 inhibiting viral template
<https://mc06.manuscriptcentral.com/cjc-pubs>

Table 1. HIVRT inhibitory activity of some natural antifungal proteins

Protein	Source	IC₅₀	Ref.
Lyophyllin	<i>Lyophyllum Shimeji</i>	7.9 nM	13
Velutin	<i>Flammulena velutipes</i>	360 µM (100% inhibition)	14
Ascalin	<i>Allium ascalonicum</i>	10 µM	15
Chitinase like protein	<i>Acacia confuse</i>	10 µM	16
Homo dimeric protein	<i>Peganum harmala</i>	1.26 µM	17

Draft

Table 2. Naturally occurring, cationic antifungal peptides

Name of antifungal peptide	PDB ID	Source of peptide	Sequence	Ref no.
Cm-p5	2MP9	<i>Cenchritis muricatus</i> (Sea snail)	SRSELIVHQRLF	18
Dermaseptin	2DCX	<i>Phyllomedusa sauvagii</i> (Waxy monkey tree frog)	ALWKTLLKKVLKA	19
Tachykinin	1N6T	<i>Gallus gallus</i> (Red jungle fowl)	HKTDSFVGLM	20
Tritrpticin	2I1D	<i>Sus scrofa</i> (Wild boar)	VRRFPWWPFLRR	21
Indolicidin variant (CP-11)	1QXQ	<i>Bos taurus</i> (Cattle)	ILKKWPWWPWRK	22

Draft

Table 3. Glide energies, MMGBSA binding energies and their respective contributions* for various antifungal peptide-viral template complexes

Antifungal peptides	Docking score	Glide energy	Glide evdw (vander Waals Contribution)	Glide ecoul (Coulombic Contribution)	MMGBSA Binding energy of the complex	Coulomb binding energy	Covalent Binding energy	Hydrogen bonding energy	Lipophilic energy	Pi-Pi packing energy	Self-contact correction	Generalized born electrostatic solvation energy	Van der Waals energy
Cm-p5	-12.52	-110.61	-35.11	-75.40	-28.06	-781.99	12.48	0.14	-0.17	-0.06	-0.61	793.12	-50.98
Dermaseptin	-7.78	-120.46	-36.41	-84.05	-23.41	-1130.10	15.99	0.28	6.75	0.00	-0.01	1140.88	-57.19
Tachykinin	-10.13	-98.93	-37.64	-61.29	-46.13	-432.17	13.01	0.46	3.42	0.00	0.13	404.65	-35.63
Tritrpticin	-13.48	-99.59	-23.76	-75.83	-46.45	-1251.24	7.71	0.24	-4.49	5.34	0.00	1240.07	-44.09
Indolicidin	-10.70	-124.38	-43.37	-81.01	-56.40	-1620.68	10.87	0.09	7.65	11.07	0.00	1580.99	-46.399
DNA primer	-	-	-	-	-129.02	6284.45	14.48	0.00	0.00	0.00	0.00	-6307.98	-119.97
Designed compound 1 (mutated indolicidin)	-9.01	-88.25	-23.08	-65.17	-38.07	-1010.90	6.39	2.13	1.80	0.00	0.00	994.11	-31.61
Designed compound 2 (mutated indolicidin with(-CH₂NH-) backbone)	-9.97	-100.75	-25.29	-75.47	-33.67	-760.30	1.42	0.00	11.24	0.00	0.00	747.40	-33.43
Designed compound 3 (mutated indolicidin with(-COO-) backbone)	-11.26	-105.44	-31.42	-74.02	-12.85	-783.03	13.396	0.00	5.75	0.00	0.00	790.66	-39.630

*All energies are in kcal/mol

Table 4. Contact analysis for docked antifungal peptides

Antifungal peptide	Peptide ligand sequence and interacting residue (in red)	Template* interacting region
Cm-p5	SRSELIVHQR ^L LF SRSELIVHQR ^L LF SRSELIVHQR ^L LF SRSELIVHQR ^L LF SRSELIVHQR ^L LF SRSELIVHQR ^L LF SRSELIVHQR ^L LF SRSELIVHQR ^L LF	Backbone and base,G22,G23 Backbone,A21,U20 Backbone,G22, G23 Backbone,G23 Backbone,A16 Backbone,A13, A14(base) Backbone,A15(base) Backbone, U14,A15(base)
Dermaseptin	XALWKTLLKKVLKA XALWKTLLKKVLKA XALWKTLLKKVLKA XALWKTLLKKVLKA XALWKTLLKKVLKA	Backbone A21, G22(base), Backbone U14(base), A15 Backbone A12 Backbone A10,C11,A12 Backbone A10
Tachykinin	HKTDSFVGLM HKTDSFVGLM HKTDSFVGLM HKTDSFVGLM HKTDSFVGLM HKTDSFVGLM HKTDSFVGLM HKTDSFVGLM	Backbone and base G22, G23 Backbone A19, U20 Backbone G22, G23 Backbone A19 Backbone U18 Backbone C17 Backbone A16 Backbone A16
Tritrpticin	VRRFPWWPFLRR VRRFPWWPFLRR VRRFPWWPFLRR VRRFPWWPFLRR	Backbone C11, A12, A13 Backbone C9,A10 Backbone 3DR5,G6 Backbone A4
Indolicidin	ILKKWPWWPWRRK ILKKWPWWPWRRK ILKKWPWWPWRRK ILKKWPWWPWRRK ILKKWPWWPWRRK ILKKWPWWPWRRK	Backbone U14 Backbone U14 Backbone A15 Backbone A19,U20(base),A21 Backbone and base G21, G22 Backbone A19

*Template sequence

5'(pApUpGpA4p3DR5pG6pGpCpCpApCpA12pA13pUpApApCpU18pA19pUpApGpGpC24pA25pUpAp) 3'

Table 5. ADME properties* of antifungal peptides and designed peptidomimetic compounds

Antifungal Peptides	Molecular weight	Solvent accessible surface area Unit Å²	Number of hydrogen bond donor	Number of hydrogen bond acceptor	Globularity	Hydrophobicity	% Oral absorption	Number of violations of Lipinski's rule
Cm-p5	1483.732	2116.350	18.750	34.650	0.611	-7.113	0	3
Dermaseptin	1708.288	2562.475	16.250	30.950	0.576	1.589	0	3
Tachykinin	1118.313	1728.613	8.000	24.900	0.637	-2.563	0	3
Tritrpticin	1901.293	2579.581	25.500	35.000	0.584	-0.889	0	3
Indolicidin	1879.327	2507.674	24.500	34.000	0.596	-1.137	0	3
Designed compound 1 (mutated indolicidin)	1314.512	1937.908	17.000	28.500	0.618	-5.941	0	3
Designed compound 2 (mutated indolicidin with(-CH₂NH-) backbone)	1244.594	2119.563	21.500	28.000	0.576	-5.391	0	3
Designed compound 3 (mutated indolicidin with(-COO-) backbone)	1319.436	2018.986	15.750	29.750	0.598	-3.338	0	3

***Permissible range:**Molecular Weight 130 - 725, Total SASA 300 - 1000 Å², 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