Rational design of novel peptidomimetics against influenza a virus: biological and computational studies

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https://doi.org/10.17952/37EPS.2024.P2258

INTRODUCTION

Influenza is a highly contagious, acute respiratory illness, which represents one of the main plagues worldwide. Even though some antiviral drugs are available, the alarming increase of virus strains resistant to them, highlights the need to find new antiviral compounds.[1]

Pep.	Sequence	IC_{50} (nM) A/Parma/24/09 H1N1
1	SLDC	6 ± 1.23
2	SKHS	1.5 ± 0.71

Table 1. HI activity of peptides **1-2**.

Previously, Superti et al. have deeper investigated the mechanism of the anti-influenza virus effect of bovine Lactoferrin (bLf) and the role of its tryptic fragments (the N and C-lobes) in the antiviral activity.[2] In particular, they evaluated the influence of bLf on hemagglutinin-mediated functions. Recently, through a truncation library, we identified the tetrapeptides, SKHS (**1**) and SLDC (**2**), derived from Blf C-lobe fragment 418-429, which were able to bind hemagglutinin (HA) and inhibit cell infection in a concentration range of femto- to picomolar (*table 1, 2*).[3]

Pep.	A/Parma/24/09 H1N1 EC_{50} (pM)	SI
1	4.6±0.05	$>5.4.10^6$
2	0.048±0.0012	$>5.2.10^8$

Table 2. I*n vitro* antiviral activity against influenza virus infection of peptides **1-2**.

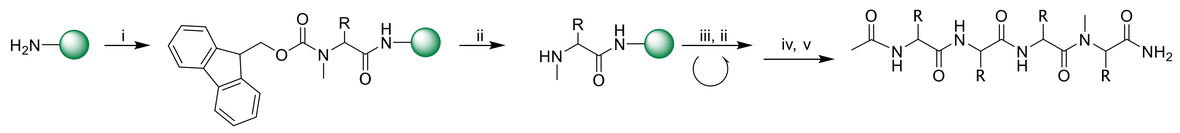
DESIGN

It is well known that the direct application of peptides as medicinal entities has some severe limitations, including high degradation by proteolytic enzymes and poor cell mem-brane permeability. We designed new peptidomimetics starting from tetrapeptide sequences SLDC (**1**) and SKHS (**2**). To increase metabolic stability and improve bioavailability, receptor affinity, and selectivity to overcome these drawbacks. Two different strategies were used to reduce the conformational freedom of the peptide backbone and increase the affinity towards the target: N-methylation and synthesis of peptoids.[4,5] Then, we carried out binding affinity measurements by microscale thermophoresis (MST) and hemagglutination inhibition assay on peptide synthesized.

CHEMISTRY

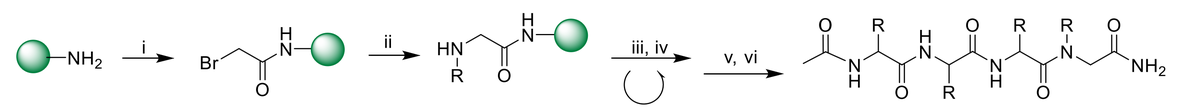
Peptides synthesis was performed according to the solid phase approach. The crude peptides were purified by preparative RP-HPLC and purity monitored by analytical HPLC. Molecular weights were confirmed by ESI-MS.

Synthesis of N-Methyl peptides



Scheme 1. General synthesis of N-methylated peptide. Reagent and conditions: (i) Fmoc-N-Me-aa(PG)-OH (3 eq.), OxymaPure (3 eq.), DIC (6 eq.), 5 min, MW 75 °C; (ii) piperidine/DMF (2:8, v/v), 1 min, MW 90 °C; (iii) Fmoc-L-aa(PG)-OH (3 eq.), OxymaPure (3 eq.), DIC (6 eq.), 2 min, MW 90 °C; (iv) Ac_2O/DCM (1:3, v/v) 2 min, MW 65 °C; (v) TFA/TIS/H_2O (95:2.5:2.5 v/v).

Synthesis of peptoids



Scheme 2. General synthesis of peptoids. Reagent and conditions: (i) bromoacetic acid/DMF (2M) and DIC (2.4 M), 5 min, MW 75 °C; (ii) R-NH_2/DMF (1M), 5 min, MW 75 °C; (iii) Fmoc-L-aa(PG)-OH (3 eq.), OxymaPure (3 eq.), DIC (6 eq.), 2 min, MW 90 °C; (iv) piperidine/DMF (2:8, v/v) 1 min, MW 90 °C; (v) Ac_2O/DCM (1:3, v/v) 2 min, MW 65 °C; (vi) TFA/TIS/H_2O (95:2.5:2.5 v/v).

RESULTS and DISCUSSION

We decided to investigate the direct binding of peptides **1**, **2**, and the peptidomimetics library to HA protein by two complementary techniques: microscale thermophoresis and surface plasmon resonance.

MicroScale Thermophoresis analysis

The 16 compounds were subjected to MST screening and the results are shown in Table 3. According to analysis, every peptide interacts with HA with different dissociation constants. Compounds **4**, **9**, **16**, and **18** bind HA more effectively than compounds **1** and **2**, showing an equilibrium dissociation constant (K_D) value in a two-digits nanomolar concentration range (figure 1).

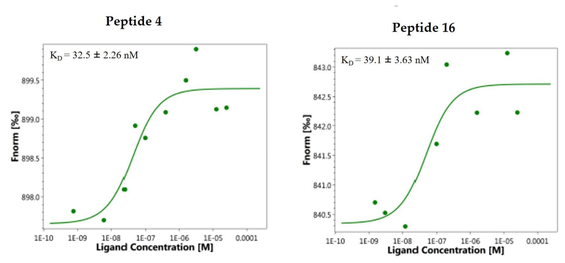


Figure 1. MST binding curve of peptides **4** and **16** to HA.

Pep.	Sequence	MST K_D (μM)	SPR K_D (μM)
1	SLDC	10.4 ± 0.23	7.12 ± 0.26
2	SKHS	7.26 ± 0.06	4.53±0.08
3	(N-Me)SLDC	24.8±0.71	7.60±2.69
4	S(N-Me)LDC	0.0325±0.002	0.58±0.34
5	SL(N-Me)DC	8.8±0.23	NPD
6	SLD(N-Me)C	0.142±0.03	3.80±1.06
7	(N-Me)SKHS	0.336±0.02	4.19±0.86
8	S(N-Me)KHS	7.93±0.73	8.33± 1.73
9	SK(N-Me)HS	0.0528±0.005	1.26±0.36
10	SKH(N-Me)S	3.04±0.85	NPD
11	*Nh*SLDC	0.0672±0.002	6.55±1.52
12	S*N*LDC	0.701±0.03	3.81±2.13
13	SL*N*DC	1.34±0.02	NPD
14	SLD*Nh*C	0.0459±0.01	NPD
15	*Nh*SKHS	7.4±0.35	2.09±2.41
16	S*N*KHS	0.0391±0.003	1.70±0.13
17	SK*N*HS	3.07±0.79	NPD
18	SKH*Nh*S	0.0491±0.001	0.43±0.21

Table 3. Sequence and binding affinities between HA and peptides **1-18** determined using MST and SPR.

SPR-based binding assays

The affinity of peptidomimetics for HA was determined by SPR. SPR study revealed that synthesized peptides effectively interact with the immobilized protein. The sensorgrams of the compounds **4** and **18** bound to HA in HBS-P buffer are shown in Figure 2. Interestingly, these peptidomimetics bind HA with higher efficiency with respect to **1** and **2**, showing a K_D value in a three-digits nanomolar concentration range.

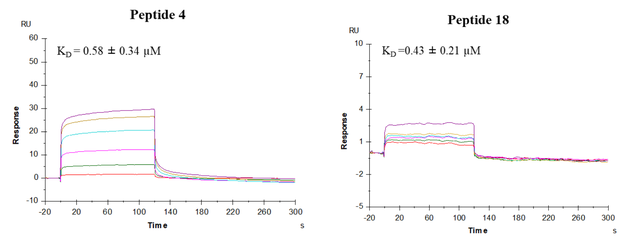


Figure 2. Sensorgram of compound **4** and **18**. Each compound was injected at four different concentrations (from 1 μM to 20 μM).

Hemagglutination assay

The capability of compounds **4**, **9**, **16**, and **18** to inhibit the hemagglutinating activity was evaluated by HI. The inhibitory activity of peptides **1** and **2** is shown as a reference. The oseltamivir-resistant A/Parma/24/09 H1N1 virus strain was used. As shown in Table 4, compounds **4** and **16** prevented the hemagglutination of the oseltamivir-resistant strain A/Parma/24/09 H1N1 at quite similar concentrations than the reference peptides.

Pep.	Sequence	IC_{50} (nM) A/Parma/24/09 H1N1
1	SLDC	6 ± 3
2	SKHS	1.5 ± 0.7
4	S(N-Me)LDC	4.6 ± 2.3
9	SK(N-Me)HS	3.2 ± 1.6
16	S*N*KHS	1.2 ± 0.6
18	SKH*Nh*S	3.3 ±1.7

Table 4. Sequence and HI activity of peptides **1**, **2**, **4**, **9**, **16**, **18**.

Neutralization assay

Next, we evaluated the ability of the four compounds to inhibit viral infection by neu-tralization assay. All compounds were able to prevent viral infection in a dose range of roughly 50 to 140 pM, corresponding to selectivity indices ≈ $10^3/10^5$. In particular, com-pound 4 inhibited the virus replication at a concentration similar to that of the reference peptide (Table 5).

Pep.	Sequence	EC_{50} (μM)	SI
1	SLDC	$4.6 \pm 0.05 \times 10^{-6}$	$>5.4 \times 10^6$
2	SKHS	$4.8 \pm 0.12 \times 10^{-8}$	$>5.2 \times 10^8$
4	S(N-Me)LDC	$8.5 \pm 0.04 \times 10^{-5}$	$>2.9 \times 10^5$
9	SK(N-Me)HS	$1.425 \pm 0.02 \times 10^{-4}$	$>1.78 \times 10^3$
16	S*N*KHS	$4.7 \pm 0.02 \times 10^{-4}$	$>5.3 \times 10^4$
18	SKH*Nh*S	$5 \pm 0.04 \times 10^{-5}$	$>5 \times 10^5$

Table 5. Sequence and Nt activity of peptides and peptoids.

Computational study

To get more clues on the tetrapeptide interaction with studied HAs, a structure-based computational analysis was carried out. Compound **4** is the derivative of SLDC where the Leu2 NH is methylated. Its docked interactions are driven by the hydrophobic contact of the Leu2 side chain in the most lipophilic region of the RBS. To satisfy this contact, the ligand inverts its binding mode with respect to the hit SLDC.

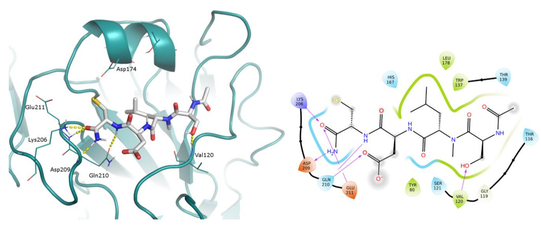


Figure 3. Docked poses of N-methyl peptide **4** represented as sticks (light orange and white C atoms, respectively) in the RBS of A/Parma/24/09 H1N1 (deep-cyan cartoon).

CONCLUSION

In this paper, we synthesized a library of peptidomimetics, N-methyl peptides and peptoids, starting from the most promising tetrapeptides previously identified.[3] Direct binding assays were carried out by two biophysical techniques (MST and SPR). Both demonstrated specific binding between compounds **4**, **9**, **16**, and **18** and HA. Therefore, the antiviral activity of these compounds was assessed through biological assays, such as HI and NT. Results from hemagglutination inhibition assay confirmed the specific interaction with HA. The neutralization test showed that S(N-Me)LDC (**4**) and SKH*Nh*S (**18**) are the most active derivatives. Computational analysis allowed us to envision the putative interactions of these ligands with HA, where they establish conserved interactions with residues of the RBS as already observed for peptides **1** and **2**.[3]
All applied methods agreed upon the identification of N-methyl peptide, S(N-Me)LDC, able to bind hemagglu-tinin with high affinity and inhibit Influenza virus hemagglutination and cell infection at nano- and picomolar concentration, respectively. This small sequence can represent a valuable starting point for the design of small molecules.[6]

References

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