Bioorganic & Medicinal Chemistry Letters 27 (2017) 4710–4713

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of peptide-derived TSLP inhibitors

Seonghu Park, Yeeun Park, Sang-Hyun Son, Kiho Lee, Yong Woo Jung, Ki Yong Lee, Young Ho Jeon *, Youngjoo Byun *

College of Pharmacy, Korea University, 2511 Sejong-ro, Sejong 30019, South Korea

ARTICLE INFO

Article history:
Received 21 July 2017
Revised 31 August 2017
Accepted 1 September 2017
Available online 7 September 2017

Keywords:
Allergic disease
TSLP inhibitors
Peptides
TSLP receptors

ABSTRACT

Thymic stromal lymphopoietin (TSLP) is a type II cytokine which is associated with most inflammatory allergic disorders in humans. It is produced mainly by epithelial cells with important role in the development of chronic inflammatory diseases by activating T-helper cell type-2 (T_H2) pathways. In this study, a total of 16 peptides were prepared by solid phase peptide synthesis based on amino acid sequences of the interface between TSLP and TSLP receptor. Their TSLP inhibition activities were determined by ELISA assay. Among them, three peptides (**6**–**8**) exhibited >50% inhibition at concentration of 0.3 mM. They can be used as hit compounds for developing peptide-based TSLP inhibitors.

Allergic disease has increasingly emerged as a significant global health issue that affects millions of individuals worldwide.[1] Allergic disease is caused by unrestrained T helper 2(T_H2)-biased immune responses that induce asthma, atopic dermatitis, and allergic rhinitis.[2,3] It has a major social and economic impact on patients, patients' family, and the society as a whole.[4] Despite being one of the most prevalent among all human disease categories, there is no effective vaccine or therapeutics to treat allergic diseases fundamentally. Therefore, understanding immune mechanisms involved in allergic diseases and finding treatment strategies for these diseases fundamentally are urgently needed.

Thymic stromal lymphopoietin (TSLP) is a cytokine belonging to interleukin 2 (IL-2) family. It is expressed mostly by epithelial cells on barrier surfaces such as the skin, lung, and gut in response to external stimuli.[5–7] TSLP is a key initiator of STAT5-mediated T_H2 inflammatory pathways.[3,8–10] It exerts biological functions by making triplex structure with an interleukin-7 (IL-7) receptor α chain (IL-7Rα) and a unique TSLP receptor (TSLPR).[11,12] TSLP alone binds to IL-7Rα with comparatively low affinity ($K_d = 2.3\ \mu M$ in mouse and no affinity was detected at 100 nM of IL-7Rα in human). TSLP-mediated T_H2 signaling is explained by sequential and cooperative formation of the ternary complex (TSLP/TSLPR/IL-7Rα). The TSLP-TSLPR binary complex ($K_d = 58$ nM in mouse and $K_d = 32$ nM in human) is formed first and then establishes the stable ternary complex with IL-7Rα.[13] Heterodimerization of TSLP with TSLPR enhanced its binding affinity for IL-7Rα ($K_d = 1.5$ nM in mouse and 29 nM in human).[13,14] Forming a triple complex of TSLP/TSLPR/IL-7Rα activates JAK1 and JAK2, thereby resulting in phosphorylation of STAT5.[15]

Numerous reports have highlighted the fact that aberrant TSLP signaling is closely associated with inflammatory allergic diseases, including asthma, atopic dermatitis, chronic obstructive pulmonary disease (COPD), and eosinophilic esophagitis.[8,9,16–18] For instance, high TSLP expression levels are known to be correlated with the severity of allergic diseases in human and mice.[3,9,19] Another study has shown that blocking TSLPR in a primate animal model can result in resistance to the development of allergic inflammation.[20] Recently, strategy for targeting TSLP, IL-25, and IL-33 together has demonstrated therapeutic potential in mouse disease models of inflammation and fibrosis.[21] Moreover, blocking TSLP signaling with an anti-TSLP monoclonal antibody is under investigation in a clinical trial to treat asthma patients.[22] These results suggest that TSLP represents a key mediator in the pathogenesis of allergic disease. Therefore, blocking TSLP signaling is considered as an attractive intervention strategy to allergic diseases fundamentally.

Identifying potent and selective inhibitors of TSLP as pharmacological tools to better understand their roles in allergic inflammatory response and their potential as therapeutics of allergic disease has been one of our research interests. Recently, Savvides and co-workers have reported the first high-resolution X-ray structure of mouse and, more recently, human TSLP/TSLPR/IL-7Rα complexes.[13,14] The 3D X-ray crystal structure of TSLP/TSLPR/IL-7Rα complex provides insight into its structure-biological function relationship. It assists in the design of TSLP inhibitors for drug

---

* Corresponding authors.
E-mail addresses: yhjeon@korea.ac.kr (Y.H. Jeon), yjbyun1@korea.ac.kr (Y. Byun).

http://dx.doi.org/10.1016/j.bmcl.2017.09.010
0960-894X/© 2017 Elsevier Ltd. All rights reserved.

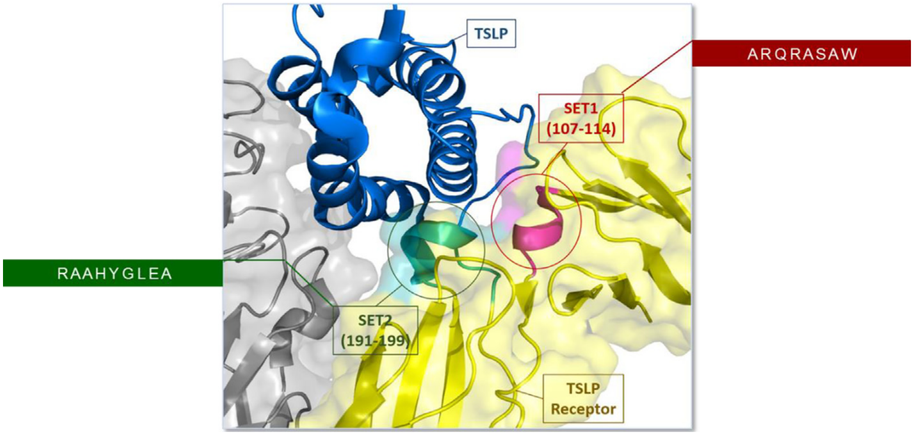


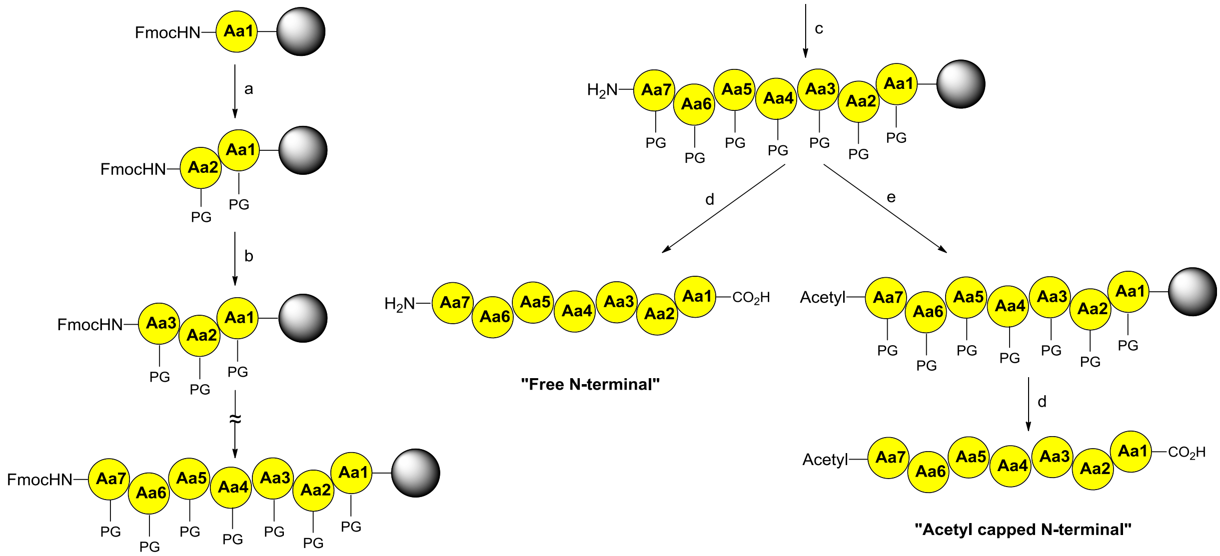
Fig. 1. Design of peptide-derived hTSLP inhibitors based on amino acid sequences of mTSLPR in the interface site between mTSLP and mTSLPR. SET 1 and SET 2 consist of ARQRASAW and RAAHYGLEA sequences, respectively.

development to treat allergic diseases. Since there has been no report on TSLP inhibitors based on peptides or small molecules, the objective of this study was to discover novel peptide-based TSLP inhibitors, by utilizing structural information of the interface between TSLP and TSLPR.

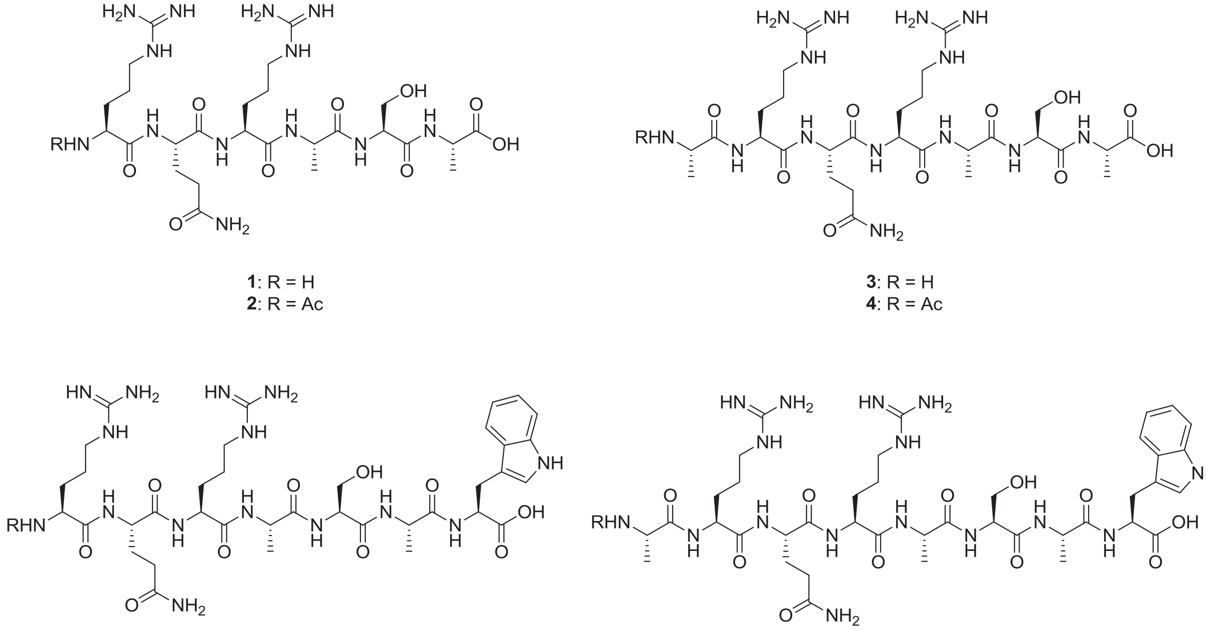
Previously, it has been reported that mouse TSLP (mTSLP) can interact with mouse TSLPR (mTSLPR) and mouse IL-7Rα heterodimer complex via two equally extensive interaction interfaces.[13] Furthermore, binding of mTSLP to mTSLPR has been characterized by the formation of two specific interaction sites: amino acid residues of 107-114 (SET 1) and 191-199 (SET 2) of mTSLPR (Fig. 1). Based on these interactions between mTSLP and mTSLPR, our hypothesis was that mimicking these amino acid residues of these specific interaction sites could disrupt the interaction between TSLP and TSLPR. As an initial step of peptide-based inhibitor design, we have exploited the mTSLPR sequence because there was the only mTSLP-mTSLPR structure available in Protein Data Bank (PDB ID: 4NN5) when we designed this experiment.[13] The sequence identity between hTSLPR and mTSLPR was 36.1%, and the similarity was 49.2%. The corresponding sequences of SET1 and SET2 in human TSLPR are ASRWNVYY and MEDVYGPDT, respectively.

Peptide-based inhibitors of protein-protein interactions (PPI) have several advantages, including high structural similarity to fragments of target protein, facile synthesis by using solid-phase peptide synthesis (SPPS), and opportunity to modify peptide sequences with various functional groups.[23] Even small linear peptides by mimicking epitopes of TSLP-TSLPR interaction sites can serve as a starting point for the design of TSLP inhibitors. Herein, we first report the synthesis of peptide-based TSLP inhibitors using SPPS and evaluation of their biological activities using ELISA assay.

To verify our hypothesis, peptides (**1**–**8**) derived from SET 1 and peptides (**9**–**16**) from SET 2 were synthesized. SPPS was applied in the synthesis of target peptides by using Fmoc-protected amino acid resin as starting materials (Scheme 1). Peptide coupling reactions were performed by using HBTU (3.0 eq)/HOBt (2.0 eq)/DIPEA (6.0 eq) in DMF for 1 h. Fmoc group in each step was removed by applying 20% piperidine in DMF for 20 min. Acetylation of *N*-terminal amine group was accomplished by treating acetic anhydride and DIPEA in DMF at room temperature. In the final step of



Scheme 1. Synthesis of peptide-derived TSLP inhibitors through solid-phase peptide synthesis (SPPS). Reagents and condition: (a) (i) 20% piperidine/DMF, 20 min; (ii) Fmoc-Aa2-OH, HBTU, DIPEA, HOBt, DMF, 1 h; (b) (i) 20% piperidine/DMF, 20 min, rt; (ii) Fmoc-Aa3-OH, HBTU, DIPEA, HOBt, DMF, 1 h; (c) 20% piperidine/DMF, 20 min; (d) TFA/thioanisole/H_2O/TIS (95:2:2:1, v/v/v/v), 3 h; (e) Ac_2O, DIPEA, DMF, 1 h.



1: R = H
2: R = Ac

3: R = H
4: R = Ac

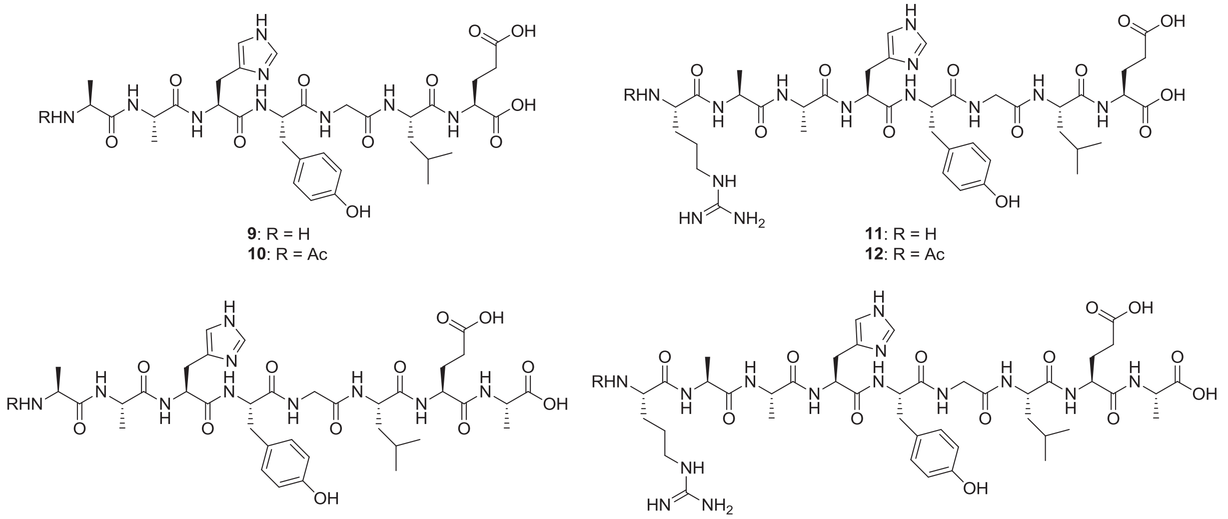
5: R = H
6: R = Ac

7: R = H
8: R = Ac

Fig. 2. Chemical structures of peptide-based inhibitors targeting amino acid residues of SET1 of mTSLPR.

syntheses, global deprotection and removal of resin were successfully performed by using a cocktail solution of TFA/thioanisole/water/triisopropylsilane(TIS) (95:2.0:2.0:1.0, v/v) for 3 h. Final products were purified using semi-preparative reversed-phase HPLC with a linear gradient condition (10% acetonitrile/90% water to 50% acetonitrile/50% water over 30 min, flow rate of 2.0 mL/min). Sixteen novel peptide-based TSLP inhibitors were prepared with overall yield of 9–26%. Final structures of these prepared peptides were fully confirmed by ^{1}H, ^{13}C NMR, ESI-MS, and HPLC (>95% purity) (See Supporting information).

Inhibition of the TSLP-TSLPR interaction by the synthesized peptides (Figs. 2 & 3) were determined by enzyme-linked immunosorbent assay (ELISA).[24] Vectors expressing hTSLP with *N*-terminal FLAG tag (FLAG-hTSLP) and hTSLPR with *C*-terminal octa-histidine tag (hTSLPR-his) were constructed. Level of binding between TSLP-tagged and TSLPR-tagged was measured by optical density (OD) at three concentrations (0.1, 0.3 and 1 mM). OD value for solution with only TSLP-tag treatment was used as control (Table 1). Among SET 1 analogues, we investigated the effect of *N*-terminal alanine and *C*-terminal tryptophan. Attachment of alanine to the core sequence RQRASA at the *N*-terminus (the peptide **3**) increased slightly inhibition activity while that of tryptophan at the *C*-terminus (the peptide **5**) increased 2 times more inhibition effect. Acetylation at the *N*-terminus also increased TSLP inhibition. In particular, peptides **6**–**8** containing tryptophan (W) in the *C*-terminus exhibited more than 50% inhibition at concentration of



9: R = H
10: R = Ac

11: R = H
12: R = Ac

13: R = H
14: R = Ac

15: R = H
16: R = Ac

Fig. 3. Chemical structures of peptide-based inhibitors targeting amino acid residues of SET2 of mTSLPR.

Table 1
TSLP inhibition for the synthesized peptides.

	Compound	Sequence	Inhibition (%)[a]		
			0.1 mM	0.3 mM	1 mM
	Control	–	0	–	–
	FLAG tag-free TSLP[b]	–	–	69 ± 1	–
SET1	**1**	RQRASA	19 ± 3	15 ± 1	18 ± 1
	2	Ac-RQRASA	22 ± 1	22 ± 2	18 ± 1
	3	ARQRASA	30 ± 1	32 ± 3	30 ± 1
	4	Ac-ARQRASA	30 ± 1	30 ± 2	26 ± 1
	5	RQRASAW	38 ± 2	47 ± 1	46 ± 2
	6	Ac-RQRASAW	38 ± 1	44 ± 1	56 ± 1
	7	ARQRASAW	42 ± 1	49 ± 2	60 ± 3
	8	Ac-ARQRASAW	50 ± 2	56 ± 3	65 ± 3
SET2	**9**	AAHYGLE	2 ± 1	3 ± 1	16 ± 3
	10	Ac-AAHYGLE	4 ± 2	11 ± 2	37 ± 4
	11	RAAHYGLE	4 ± 1	10 ± 1	45 ± 3
	12	Ac-RAAHYGLE	5 ± 1	15 ± 1	52 ± 3
	13	AAHYGLEA	5 ± 2	23 ± 2	59 ± 1
	14	Ac-AAHYGLEA	2 ± 2	4 ± 1	26 ± 4
	15	RAAHYGLEA	7 ± 2	17 ± 2	35 ± 5
	16	Ac-RAAHYGLEA	12 ± 7	11 ± 4	34 ± 3

[a] Measurements were performed in triplicate and presented as mean ± SD of at least three experiment sets.
[b] The concentration of FLAG tag-free TSLP was 1 μg/mL.

1 mM, suggesting that the bulky and hydrophobic tryptophan at the side chain contributed to the binding of these peptides to the TSLP. Compound **8** with tryptophan at the *C*-terminus and acetyl group at the *N*-terminus was the most potent one. It showed 50% inhibition at concentration as low as 0.1 mM. Among SET 2 analogues, compounds **12**–**13** showed relatively strong inhibition compared to the other peptides. However, the SET2-based peptides were less potent than the SET1-based ones.

In conclusion, sixteen peptide-derived ligands were designed as hTSLP inhibitors based on the X-ray crystal structure of TSLP/TSLPR/IL-7Rα complex. These peptides were prepared by applying solid phase with moderate yields. Results by ELISA showed that SET1-derived peptides had relatively stronger binding affinity for TSLP compared to SET2-derived peptides. Among SET1 analogues, compounds **6**–**8** with hydrophobic tryptophan at the side chain were found to be more potent than the other peptides. Compound **8** possessing lipophilic tryptophan at the *C*-terminal and acetyl group at the *N*-terminal was the most potent TSLP inhibitor of this series. It inhibited the interaction between TSLP and TSLPR with a higher than 50% inhibition at 0.1 mM concentration, indicating that it has a potential to be used as a hit compound for the identification of peptide-based TSLP inhibitors.

Acknowledgments

This research was supported by a grant (2014R1A4A1007304) of the National Research Foundation (NRF), Republic of Korea.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.09.010.

References

1. Larsen JN, Broge L, Jacobi H. *Drug Discov Today*. 2016;21:26–37.
2. Holgate ST, Polosa R. *Nat Rev Immunol*. 2008;8:218–230.
3. Ziegler SF, Artis D. *Nat Immunol*. 2010;11:289–293.
4. Julia V, Macia L, Dombrowicz D. *Nat Rev Immunol*. 2015;15:308–322.
5. Sims JE, Williams DE, Morrissey PJ, et al. *J Exp Med*. 2000;192:671–680.
6. Reche PA, Soumelis V, Gorman DM, et al. *J Immunol*. 2001;167:336–343.
7. Quentmeier H, Drexler HG, Fleckenstein D, et al. *Leukemia*. 2001;15:1286–1292.
8. Ziegler SF. *J Allergy Clin Immunol*. 2012;130:845–852.
9. Ziegler SF, Roan F, Bell BD, Stoklasek TA, Kitajima M, Han H. *Adv Pharmacol*. 2013;66:129–155.
10. Bell BD, Kitajima M, Larson RP, et al. *Nat Immunol*. 2013;14:364–371.
11. Park LS, Martin U, Garka K, et al. *J Exp Med*. 2000;192:659–670.
12. Pandey A, Ozaki K, Baumann H, et al. *Nat Immunol*. 2000;1:59–64.
13. Verstraete K, van Schie L, Vyncke L, et al. *Nat Struct Mol Biol*. 2014;21:375–382.
14. Verstraete K, Peelman F, Braun H, et al. *Nat Commun*. 2017;8:14937.
15. Rochman Y, Kashyap M, Robinson GW, et al. *Proc Natl Acad Sci U S A*. 2010;107:19455–19460.
16. Redhu NS, Gounni AS. *Clin Exp Allergy*. 2012;42:994–1005.
17. Noti M, Wojno ED, Kim BS, et al. *Nat Med*. 2013;19:1005–1013.
18. Siracusa MC, Kim BS, Spergel JM, Artis D. *J Allergy Clin Immunol*. 2013;132:789–801. quiz 788.
19. Zhou B, Comeau MR, De Smedt T, et al. *Nat Immunol*. 2005;6:1047–1053.
20. Cheng DT, Ma C, Niewoehner J, et al. *J Allergy Clin Immunol*. 2013;132:455–462.
21. Vannella KM, Ramalingam TR, Borthwick LA, et al. *Sci Transl Med*. 2016;8:a365.
22. Gauvreau GM, O'Byrne PM, Boulet LP, et al. *N Engl J Med*. 2014;370:2102–2110.
23. Wojcik P, Berlicki L. *Bioorg Med Chem Lett*. 2016;26:707–713.
24. ELISA assay was conducted in a Ni-NTA HisSorb plate (Qiagen, Hilden, Germany). In brief, 100 μL of a solution containing hTSLPR with *C*-terminal octa-histidine tag (TSLPR-His) was added to each well and incubated for 2 h at 25 °C. After incubation, the plate was washed twice with 200 μL of PBS with 0.05% Tween-20 to remove unbound TSLPR-His, and the synthesized peptides as well as TSLP with *N*-terminal FLAG tag (FLAG-hTSLP) were added at 100 μL each. After 16 h incubation at 4 °C, the plate was washed twice and blocked with 100 μL of blocking buffer (PBS with 0.05% Tween-20 and 1% non-fat dry milk). The plate was washed twice to remove unbound FLAG-hTSLP and then coated with 100 μL of monoclonal anti-FLAG antibody conjugated to HRP (Sigma-Aldrich Co., USA) for 2 h at room temperature. Following incubation, the plate was washed five times and further treated with 200 μL of *o*-phenylenediamine dihydrochloride (Sigma-Aldrich Co., USA) solution and incubated for 30 min. After incubation, 1N HCl was added to stop the reaction. Optical densities (ODs) were measured at 450 nm using a microplate spectrophotometer. The inhibition percentage was calculated using the following the formula: Inhibition (%) = (1 − OD of sample/OD of control) × 100.