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Research article

Bioinformatics analysis of adhesin-binding potential and ADME/Tox profile of anti-*Helicobacter pylori* peptides derived from wheat germ proteins☆

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ABSTRACT

Anti-adhesive activity of wheat germ-derived peptides, which is considered as one of the promising strategies for preventing *Helicobacter pylori* infection, was investigated. The underlying mechanism of anti-adhesive action was due to peptides acting as receptor analogues and binding to *H. pylori* adhesin proteins. However, there is lack of information on the nature and strength of this molecular interaction as well as the participating species and drug-likeness of the food-derived bioactive peptides. In this study, the biostability and ADME/Tox (absorption, distribution, metabolism, excretion and toxicity) profile of the anti-adhesive peptides were analyzed using bioinformatic tools, and their binding potential to *H. pylori*'s adhesins estimated by molecular docking. Binding is facilitated by mostly hydrogen bonding and hydrophobic interaction occurring in the active site of the adhesin proteins with affinities ranging from -6.0 to -7.4 and -6.0 to -7.8 kcal/mol for BabA and SabA, respectively. The results indicate highly possible binding capabilities of the peptides to adhesin proteins. Out of 16 peptides studied, 14 bound in the vicinity of the active site of BabA and SabA whereas two different peptides demonstrated allosteric binding. The most hydrophobic peptide, P210 showed strong binding affinity for both BabA and SabA and, therefore, predicted to be the most promising peptide for further development in the prevention, management and treatment of *H. pylori* infection. The selected peptides were shown to be non-toxic, and to have high potential of localized effect of interfering with bacterial adherence. This work provides insights into the anti-adhesive mechanism of peptides and new evidence demonstrating bioactive peptides as promising nutraceutical candidates for preventing *H. pylori* infection.

1. Introduction

Helicobacter pylori (*H. pylori*) is a pathogen responsible for gastric-related infections that affects approximately 50% of the world's population (Van Duynhoven and De Jonge, 2001). *H. pylori* has been classified as a class I carcinogen by the World Health Organization (WHO), and approximately 10–15% of those infected will develop peptic ulcer or gastric adenocarcinoma (Sun et al., 2020a). Currently, antibiotics therapy is the most widely used treatment for *H. pylori* infection; however, it has many side effects and induces the emergence of antibiotic-resistance bacteria (Narayanan et al., 2018). Therefore, it is important to explore preventive therapy and alternative treatments for *H. pylori* infection (Yonezawa et al., 2015).

The mode of infection and survival of *H. pylori* within the harsh environment of the gastric mucosa involve the use of its adhesin proteins, such as BabA and SabA, to bind tightly to the sugar moieties of Lewis^{b} and sialyl-Lewis^{x}, respectively. Their binding to the Lewis antigens on the surface of the epithelial cells lining the stomach and duodenum are mainly mediated by networks of hydrogen bonding and does not cause any conformational change in the adhesin protein (Hage et al., 2015; Pang et al., 2014). Hence, anti-adhesive therapy targeting specifically the active site of the adhesin protein is considered as one of the promising strategies for preventing *H. pylori* infection (Sun et al., 2020b). The principle of anti-adhesive therapy is the interference or inhibition of bacterial adherence to host cells by using anti-adhesive agents as receptor analogs or adhesin analogs, subsequently preventing infection (Sun and

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Table 1. Sequences of 33 bioactive peptides with gastric biostability (resistance against pepsin)*.

No.	Peptide sequence	No.	Peptide sequence
2	DAVTYTEHAR	193	RVTIMPK
4	VQASIAANTWVVSGTPQTK	207	AVVIHVPYR
16	AGGAYTMNTASAVTVR	210	VTGAIPI
36	VEIIANDQGNR	212	YYCTVIDAPGHR
42	DNIQGITKPAIR	213	KMEVPYCIVK
64	YDDMWAGWCVK	218	HTGSAGGGGISR
70	ISANIAAR	220	KAVVIHVPYR
73	PAGNVGEIR	236	KGHAVGDIPGVR
86	TIVQQVEAYR	247	RVNQAYVIATSTK
89	IISSIEQK	249	MDNNTVGGSR
90	IGGIGTVPVGR	251	VPKPAGNVGEIR
97	DNIEGITKPAIR	253	CQAIHNVAEAIR
102	DNIQGITK	255	ITRPHGNSGVVR
115	MISVTGPR	258	DASPSAMTPGAR
141	GHAVGDIPGVR	262	RQGNTARSR
165	NVYYGVAPVAQK	264	VTMVEIE
184	VPIPNPSGDR		

* The peptides were hydrolyzed *in silico* with pepsin at physiological pH 1.3 using ExPASy Peptide Cutter.

Wu., 2017). Current research mainly focuses on the identification and development of food and plant-derived receptor analogs that competitively bind bacterial adhesins (Parente et al., 2003; Niehues et al., 2010; Gottesmann et al., 2020). For example, a recent study showed that defatted wheat germ protein hydrolysates (DWGPH) exhibited anti-adhesive property against *H. pylori* infection (Sun et al., 2020a). The study suggested that this anti-adhesive property was due to DWGPH peptides binding to adhesin proteins, BabA (blood group antigen-binding adhesin A) and SabA (sialic acid–binding adhesin) of *H. pylori*. A database of 267 anti-adhesive peptides was identified from DWGPH and could be used to establish structure-activity relationships (Sun et al., 2020a). However, there is a dearth of information on molecular interactions between peptide and *H. pylori* adhesin.

Understanding the molecular interactions between peptide and adhesin using wet lab techniques is challenging because the *H. pylori* adhesins such as BabA and SabA are not commercially available and their purification is laborious, costly, and time-consuming. Molecular docking is considered a strategic approach for addressing this challenge as it could help in visualizing the interaction pattern, measuring binding energy of receptor-ligand complexes, and predicting the binding site of peptides in the adhesin proteins (Agyei et al., 2018). Also, computational methods are promising tools for investigating the toxin profile, biostability and drug-likeness of the nutraceutical candidates (Liu et al., 2006). Therefore, the objectives of this study were to use bioinformatics

Table 2. Predicted binding sites and affinities of anti-adhesive peptides with *H. pylori* adhesins BabA (PDB: 4ZH0) and SabA (PDB: 4O5J).

No.	Peptide sequence	BabA-peptide binding					SabA-peptide binding				
		Binding affinity (kcal/mol) BabA	Residues involved within 5 Å	No. of H-bonding	H-bond distance (Å)	Active torsion	Binding affinity (kcal/mol) SabA	Residues involved within 5 Å	No. of H-bonding	Binding distances	Active torsion
2	DAVTYTEHAR	-7.1	A-110, N-111*, G-112, F-122, I-150, E-151, K-154*, K-155, N-157, E-158, A-159, I-162, Y-185•, Y-187, N-196, C-197, Q-200, V-201, T-202, G-203, K-214, I-215, Q-216*, T-217, I-218, D-219, G-220, T-227, I-248, N-250*, A-264, Q-265*, T-268, L-269	6	1.9–2.5	42	-7.0	L-94, W-97*, N-103, F-105, S-131, V-122, Q-123••, G-124, Q-145, Y-148, D-149, K-152*, K-153, E-156••, D-157, L-158, Q-159, A-160, T-163, S-165, K-168, P-332, N-334, P-335, Y-336, R-337, Q-338	4	2.4–2.6	42
70	ISANIAAR	-6.7	K-77, A-78, N-79, Y-83, Q-84*, L-87, N-91, L-167, G-170, L-171, L-395, A-396, T-397, C-398, Q-410, G-411*, A-413, P-414, C-426, A-427, Y-428, V-429, G-430, Q-431, T-432, T-434, N-435*••, N438, S-439, H-442	5	2.2–2.8	29	-7.3	Y-65, S-68, F-69, P-70, N-72, T-77, T-78•, Q-79, S-80, P-81, F-83, N-84, Q-87•, T-91, Q-162, T-163, N-170, N-171, L-172, Q-338•, Q-344, E-345•, T-348, N-351**•, N-352, Y-355, Y-356, R-359	7	2.0–2.8	29
73	PAGNVGEIR	-6.9	K-114, K-119, I-121, N-123*, N-124*, E-125, S-130, T-131, S-132, T-134, Q-161, Q-164, T-165, K-168, N-182, V-183, T-184, Y-185, T-186, T-188, V-232, N-239, T-240, T-241, Y-245, E-247•, P-414, G-415••, T-416, V-417•, T-418	6	2.1–2.4	31	-7.2	L-94, W-97, S-98•, G-102, N-103, F-105, S-121, V-122, Q-123•, G-124, Y-148, K-152*, A-155, E-156, Q-159, T-163, S-165*, K-168, S-330, P-332, T-333, N-334**, P-335, R-337, Q-338	6	2.0–2.6	31
86	TIVQQVEAYR#	-7.4	N-109, A-110, N-111, G-112, S-149, I-150, E-151, K-154**, K-155*, E-158, A-159, I-162, Y-185, Y-187, N-196•, C-	11	2.0–2.7	44	-6.9	L-94, W-97, S-98, A-101, G-102, N-103, Y-104, Q-123, G-124, K-152*, K-153, E-156, D-157, L-158, Q-159*, A-	5	2.1–2.6	44

(*continued on next page*)

Table 2 (*continued*)

No.	Peptide sequence	BabA-peptide binding					SabA-peptide binding				
		Binding affinity (kcal/mol) BabA	Residues involved within 5 Å	No. of H-bonding	H-bond distance (Å)	Active torsion	Binding affinity (kcal/mol) SabA	Residues involved within 5 Å	No. of H-bonding	Binding distances	Active torsion
			197, Q-200, V-201••, T-202, G-203, K-212, K-214*, I-215•, Q-216, T-217*, I-218, D-219*, G-220, T-225, I-227, I-248, N-250, L-252, V-255, A-261, A-264, Q-265*, S-267, T-268, N-271, Q-319,					160, A-161, T-163, N-164, S-165*, A-166, K-168, N-171, N-189, S-190, N-194, L-198, K-324, P-332, N-334**, P-335, Y-336, R-337, C-338			
89	IISSIEQK	-6.3	N-109, A-110, N-111, G-112, I-150, E-151, N-152, K-154*, K-155*, L-156, E-158•, A-159, I-162, Y-185, Y-187, N-196•, V-201, K-214*, Q-216, T-217, I-218, D-219, T-225, I-227, N-250, L-252, A-261, A-264, Q-265*, S-267, T-268, N-271, T-272, N-275	6	2.0–2.5	39	-6.9	L-94, W-97, S-98, G-102, N-103, F-105, V-122, Q-123•, Y-148, D-149, K-152, K-153, A-155, E-156•, Q-159*•, A-160, Q-162, T-163•, N-164, S-165, K-168•, G-169, Y-322, K-324, P-332, N-334, P-335, Y-336, R-337, Q-338*, V-339	7	1.9–2.6	39
102	DNIQGITK	-6.5	N-109, A-110, N-111*, G-112, S-149, I-150, E-151, K-154*, K-155, E-158, A-159, I-162, Y-185, Y-187, N-196, Q-200, V-201, T-202, G-203, K-214, Q-216•, T-217, I-218, D-219, G-220, T-225, I-227, N-250, L-252, A-261, A-264, Q-265, S-267, T-268•	4	1.9–2.6	36	-6.6	T-77, T-78••, Q-79, S-80, P-81, F-83, N-84, Q-87, Q-162•, T-163, N-164, K-168•, G-169, N-170, N-171, L-172, Q-338*•, N-341*, Q-344, E-345, T-348, K-350, N-351*•, N-352•, S-354, Y-355, Y-356	10	1.9–2.5	36
115	MISVTGPR	-6.5	A-110, N-111, F-122, E-151, K-154, K-155, N-157, E-158, A-159, I-162, Y-185, Y-187, V-201, T-202, K-214, Q-216*, T-217••, I-218, I-D-219, G-220, I-227, I-248, N-250, L-252, A-261, A-264, Q-265•, T-268,	4	2.0–2.3	30	-7.0	L-94, W-97*, S-98, A-101, G-102, N-103, S-121, V-122, Q-123•, Q-145, Y-148, D-149, K-152*, A-155, E-156•, Q-159*, A-160, T-163, S-165, K-168, P-332, T-333, N-334*, P-335••, Y-336, R-337	8	1.9–2.4	30
184	VPIPNPSGDR	-6.9	G-101, Y-102••, V-103, T-104, Q-105*, C-106, G-107, K-119, I-121, N-123, N-124, G-127, Y-128*•, R-129, S-130, T-131, S-132*, I-133, T-134*••, C-135, S-136*, L-137, T-186, T-188, T-241, Y-245, E-247, Y-279, H-281,	10	1.8–2.4	32	-6.5	Q-42, A-44, S-45, Q-48, S-49*, N-52, S-61, S-62, N-64•, Y-65, L-66, S-68, N-72, Y-355, Y-356, R-359, D-361, A-362, A-363, S-365*•, R-368*, D-369, N-376, E-379	5	2.3–2.5	32
193	RVTIMPK#	-6.6	R-63, N-329, E-330•, H-331, E-332•, Q-333, T-334, T-335**, P-336, V-337, G-338, N-339, F-345, P-347, F-348•, T-349, D-350, A-351, S-352*, F-353, A-354, G-356, M-357, L-460•, V-461, N-462, F-463	7	2.2–2.5	31	-7.7	L-94, W-97*, S-98, G-102, N-103, Y-104, V-122, Q-123, G-124, N-125, K-152*, E-156, Q-159*, A-160, T-163, N-164, S-165, K-168, G-169, P-326, A-328•, G-329, S-330, T-331, P-332, T-333•, N-334*, P-335, Y-336, R-337, Q-338	6	2.1–2.4	31
207	AVVIHVPYR	-7.3	N-109, A-110, N-111, G-112, E-151, N-152, K-154*, K-155*, E-158, A-159, I-162, Y-185, Y-187•, N-196, V-201, T-	5	2.2–2.6	32	-6.9	L-94, S-98, G-102, N-103, Q-123*•, K-152**, K-153, A-155, E-156••, D-157, Q-159, A-160, T-163, S-165, K-168, A-	7	1.9–2.5	32

(*continued on next page*)

Table 2 (*continued*)

No.	Peptide sequence	BabA-peptide binding					SabA-peptide binding				
		Binding affinity (kcal/mol) BabA	Residues involved within 5 Å	No. of H-bonding	H-bond distance (Å)	Active torsion	Binding affinity (kcal/mol) SabA	Residues involved within 5 Å	No. of H-bonding	Binding distances	Active torsion
			202, K-214, Q-216, T-217, I-218, D-219, G-220, I-227, I-248, N-250, A-264, Q-265, S-267, T-268, N-271, T-272*, N-275*,					321, Y-322, P-332, T-333, N-334, P-335, Y-336*, R-337, Q-338			
213	KMEVPYCIVK	-6.5	N-109, A-110, N-111, G-112, S-149, I-150, E-151, K-154, K-155, E-158, A-159, I-162, Y-185, Y-187, N-196, Q-200, V-201••, T-202•, G-203, V-204, K-212, K-214, I-215, Q-216*, T-217, I-218, D-219, G-220*, K-221, I-227, N-250, L-252, A-264, Q-265•, T-268	6	2.0–2.6	46	-6.3	L-94, W-97, S-98, N-103, Y-104, F-105, Q-123, G-124, Y-148, K-152, A-155, E-156, Q-159, A-160, T-163, N-164, S-165, K-168, P-326, A-328, G-329, S-330•, T-331, P-332, T-333, N-334*, P-335•, Y-336, R-337, Q-338	3	1.9–2.5	46
210	VTGAIPI[#]	-7.0	G-101, Y-102•, V-103, T-104•, Q-105, C-106, K-119, I-121, N-123, G-127, Y-128, S-130*, T-131, S-132, I-33, T-134*, C-135, S-136, T-188, S-190, T-241, V-243, Y-245, E-247, H-281, A-282	4	2.1–2.4	21	-7.8	L-94, W-97, S-98, G-102, N-103, K-152*, A-155, E-156, Q-159, A-160, T-163, N-164, S-165, K-168, Y-322, K-324, P-332, T-333•, N-334, P-335, Y-336, R-337, Q-338*	3	2.1–2.4	21
220	KAVVIHVPYR	-6.7	N-109, A-110, N-111, G-112, S-149, I-150, E-151, K-154*, K-155, L-156, E-158, A-159, I-162, Y-185, Y-187, N-196, Q-200, V-201, T-202, K-214, Q-216, T-217•, I-218, D-219, G-220, T-225, I-227, N-250, L-252, A-264, Q-265, S-267, T-268, N-271, T-272, Q-319	2	1.9–2.1	39	-6.0	L-94, W-97, S-98, G-102, N-103, F-105, S-121, V-122, Q-123*, G-124, Q-145, Y-148, D-149, K-150, K-152, K-153, A-155, E-156•••, Q-159, S-165, K-168, P-332, T-333, N-334**, P-335, Y-336,	6	1.9–2.4	39
249	MDNNTVGGSR	-7.0	G-101, Y-102, V-103, T-104, Q-105, C-106*, K-119, I-121, N-123, N-124*, E-125, C-127, Y-128, R-129, S-130, T-131, S-132*, I-133, T-134*, C-135, S-136, T-186, T-188, C-189, S-190*, V-232, N-239, T-240, P-241, V-243, S-244, Y-245•, E-247, FH-281, A-282, T-419, F-422	6	2.1–2.6	40	-6.6	L-94, W-97, S-98, G-102, N-103, F-105, V-122, Q-123, G-124, N-125, Y-148*, D-149, K-152*, K-153, A-155, E-156•, D-157, Q-159, A-160, T-163, S-165, P-332, N-334*, P-335•, Y-336, R-337, Q-338	5	2.2–2.5	40
262	RQGNTARSR[#]	-6.0	A-110, N-111, I-150, E-151••, N-152, F-153, K-154, K-155, L-156, N-157, E-158•, A-159, Y-185, Y-187, S-199, Q-200••, V-201, T-202, G-203, K-214, Q-216, T-217•, I-218, D-219, I-227, N-250*•, A-261, A-264, Q-265, T-268, N-271, T-272, N-275	8	2.0–2.9	40	-6.2	E-133, N-134•, C-135, S-136, G-137, I-138, E-139, M-214, W-215, K-216, N-217, G-228•, A-229, I-230, T-231*•••, S-232, T-233*•, N-234, Q-238, Y-239, A-240*, V-241, N-243, N-244•, L-306, S-309, I-310, P-311, E-313, Q-314*	11	1.8–2.5	40
264	VTMVEIE	-6.0	N-109, A-110, N-111**, G-112, S-149, I-150, E-151, K-154*, K-155, E-158, A-159, Y-185, Y-187, N-196, S-199, Q-200, V-201•, T-202, G-203,V-204, K-212, K-	6	2.1–2.5	32	-6.8	L-94, W-97, S-98, G-102, N-103*, Y-104, F-105, Q-123, G-124, K-152*, A-155, E-156, Q-159*•, A-160, T-163, S-165, K-168, A-321, Y-322, K-324, P-332, N-	5	2.2–2.6	32

(*continued on next page*)

Table 2 (*continued*)

No.	Peptide sequence	BabA-peptide binding					SabA-peptide binding				
		Binding affinity (kcal/mol) BabA	Residues involved within 5 Å	No. of H-bonding	H-bond distance (Å)	Active torsion	Binding affinity (kcal/mol) SabA	Residues involved within 5 Å	No. of H-bonding	Binding distances	Active torsion
			214*, Q-216*, T-217, I-218, D-219, I-227, I-248, N-250, Q-265, T-268					334*, P-335, Y-336, R-337, Q-338			

[#] Selected as representative anti-adhesive peptides.
* Indicates H-bond donor which could be *,**,*** for 1,2,3 donors respectively.
• Indicates H-bond acceptor which could be •,••,••• for 1,2,3 acceptors respectively.
BabA Binding site: Vicinity of C189, G191, N194, N206, D233, S234, S244, and T246.
SabA binding site: Vicinity of S-80, P-81, W-97, Y-148, K-152, Q-159, and Q-162.

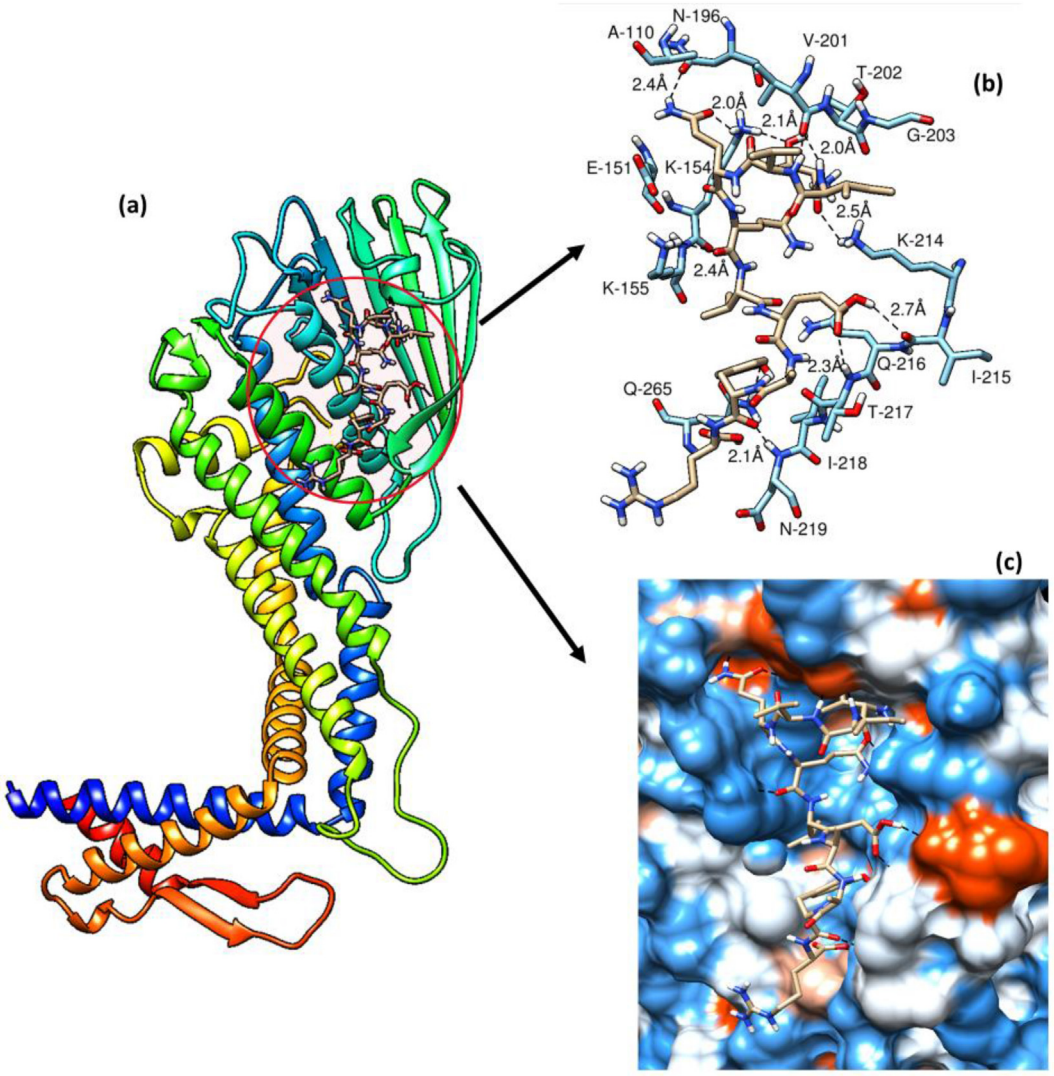

Figure 1. (a) Binding of pepsin-resistant wheat germ peptides P86 at the vicinity of the active site of *H. pylori* adhesin BabA (b) interacting amino acids within 3Å and hydrogen bond pattern (c) charge environment and hydrophobicity/hydrophilicity of the binding pocket in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white 0.0 to orange red for the most hydrophobic.

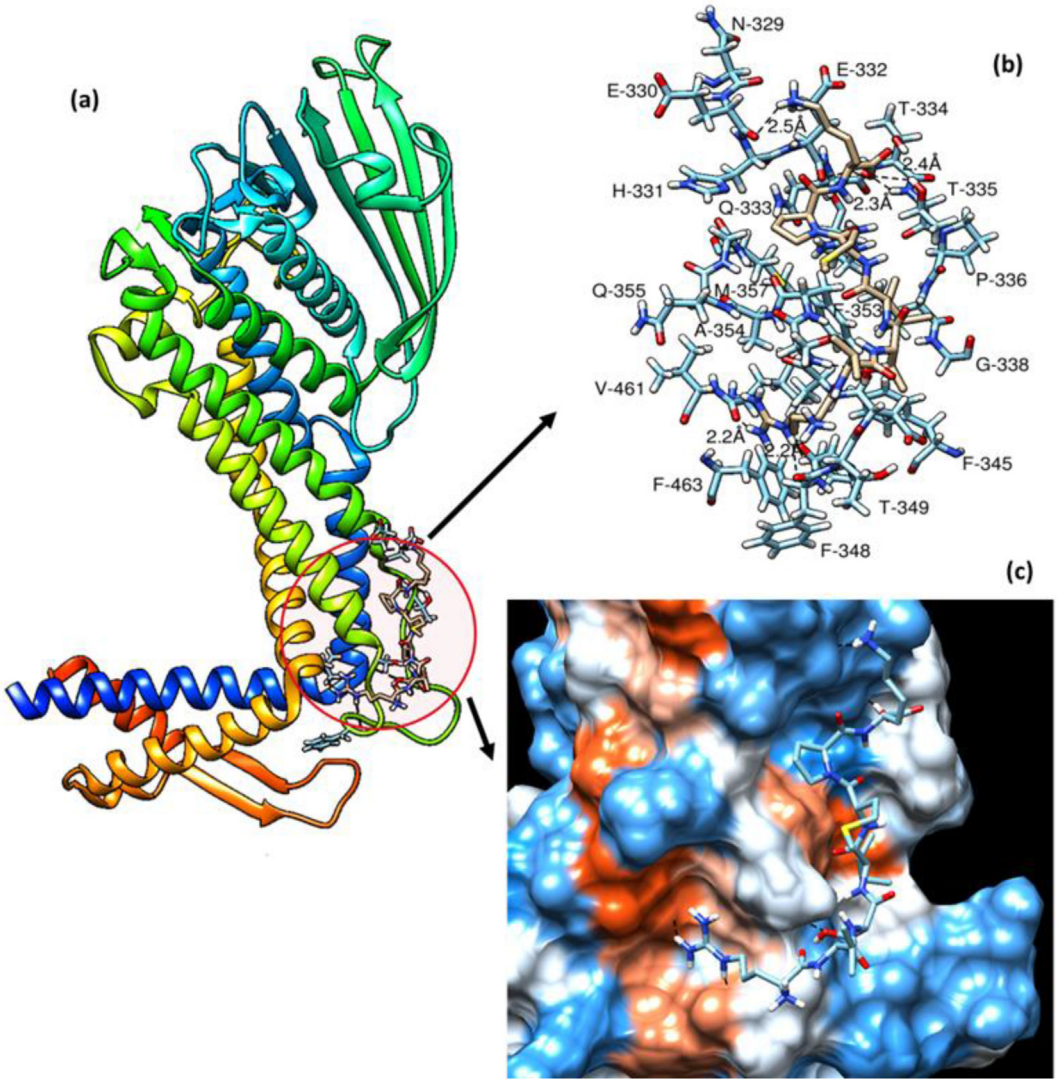

Figure 2. (a) Binding of pepsin-resistant wheat germ peptides P193 at the allosteric site of *H. pylori* adhesin BabA (b) interacting amino acids within 3Å and hydrogen bond pattern (c) charge environment and hydrophobicity/hydrophilicity of the binding pocket in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white 0.0 to orange red for the most hydrophobic.

tools to evaluate potential pepsin-resistant DWGPH peptides that bind to the active sites of BabA and SabA at high affinity, which could prevent bacteria adherence to Lewis antigens of the gastric mucosa, and to study the ADME/Tox (absorption, distribution, metabolism, excretion and toxicity) profile of the peptides.

2. Materials and methods

2.1. Anti-adhesive peptide library

A database of 267 peptides identified in defatted wheat germ protein hydrolysate with anti-adhesive property against *H. pylori* was retrieved from our recent study (Sun et al., 2020a).

2.2. Biostability assessment by PeptideCutter

The anti-adhesive peptides were hydrolysed *in silico* with pepsin using ExPASy PeptideCutter (https://web.expasy.org/peptide_cutter/). Pepsin is a stomach enzyme with optimum activity at pH 1.3. The hydrolysis tool generated a map of the peptide sequence with indication of pepsin cleavage sites and resulting fragments. Thirty-three out of the 267 peptides showed resistance against pepsin degradation in the gastric phase of digestion and therefore were chosen for subsequent evaluations.

2.3. Molecular docking of anti-adhesive peptides to adhesin proteins

Among the 33 pepsin-resistant peptides, only 16 with maximum length of 10 amino acids were selected for docking due to size constraint of the docking program. The crystal structures of the two *H. pylori* adhesins (BabA and SabA) were retrieved from the RCSB Protein Data Bank (PDB), with PDB code 4ZH0 and 4O5J, respectively. Chimera UCSF software version 1.15 (Pettersen et al., 2004) and Autodock Vina package version 1.1.2 (Trott and Olson, 2012) were used to generate peptide structures and perform molecular docking study. Polar hydrogen atoms and Gasteiger charges were added, and non-standard amino acid residues were ignored prior to docking. All structures were minimized to eliminate internal clashes and optimize structure with minimum energy. Water and ligands crystalized alongside the adhesin proteins were removed prior to docking simulation to minimize interference or blocking of the active site. The whole protein was selected as potential binding site and grid box dimensions for estimating peptide-BabA (4ZH0) binding were center: 14.3954, 12.2729, 43.3475; and size: 59.4532, 43.0248, 99.6112 while that of peptide-SabA (4O5J) binding were center: 90.697,

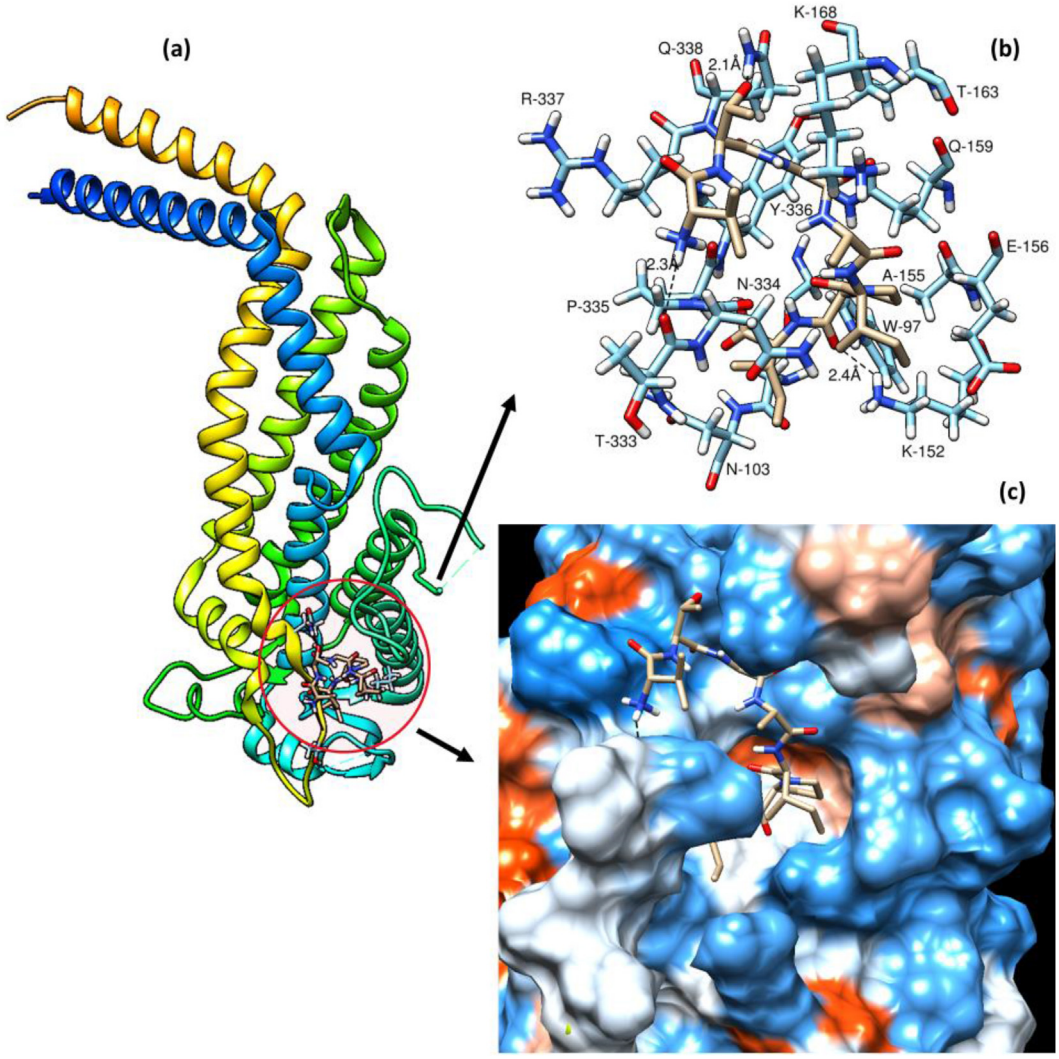

Figure 3. (a) Binding of pepsin-resistant wheat germ peptides P210 at the vicinity of the active site of *H. pylori* adhesin SabA (b) interacting amino acids within 3Å and hydrogen bond pattern (c) charge environment and hydrophobicity/hydrophilicity of the binding pocket in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white 0.0 to orange red for the most hydrophobic.

-15.156, -2.741; and size: 41.923, 90.429, 66.667. The docking scores for binding affinity values were selected as the best-ranked docking pose of peptides. Intermodel hydrogen bonds were determined by relaxing the constraints by 0.4 Å and 20°.

2.4. In silico drug-likeness evaluation

The drug-likeness of the 16 docked peptides were assessed *in silico* using SwissADME (http://www.swissadme.ch/index.php). SwissADME is a free and validated web tool that is user-friendly to support non-experts in drug discovery. SwissADME allows for the prediction and evaluation of physiochemical and pharmacokinetics (based on ADME parameters) of small molecules (Daina et al., 2017). In addition, ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/index.html) was used as a method for predicting the potential toxicity of the wheat germ protein-derived antiadhesive peptides.

3. Results and discussion

3.1. Bioactive peptides with gastric stability

DWGPH was reported to reduce adherence of *H. pylori* to a human gastric cell line, and 267 peptides were identified from the bioactive fraction that were bound to *H. pylori* (Sun et al., 2020a). This anti-adhesive property was hypothesized to be due to peptides binding to *H. pylori via* adhesins such as BabA and SabA, thus preventing *H. pylori* from binding to host cells. The peptides should not be hydrolyzed by pepsin in stomach, which could lead to the loss of binding activity and anti-adhesive activity against *H. pylori*. To select the pepsin-resistant peptides for the docking study, the initial database of 267 peptides were hydrolyzed *in silico*. As shown in Tables 1 and 3 peptides maintained intact sequences after pepsin activity *in silico*. The pepsin-resistant activity is important because the peptides must remain structurally intact in the stomach where their anti-adhesive activity is needed. The structural properties including molecular weight, pI, instability index, aliphatic index, net charge, boman index, hydrophobicity, Hmoment (α-helix), and Hmoment (β-sheet) of all peptides were analyzed in our previous work (Sun et al., 2020a,b). There are no structure-activity relationships among these 33 peptides.

3.2. Binding affinities and substantive binding sites of peptides to H. pylori's adhesins BabA and SabA

The substantive binding sites of the pepsin-resistant peptides as well as the strength and nature of their interaction with *H. pylori* adhesins, BabA and SabA, are presented in Table 2, Figures 1, 2, 3, and 4. The

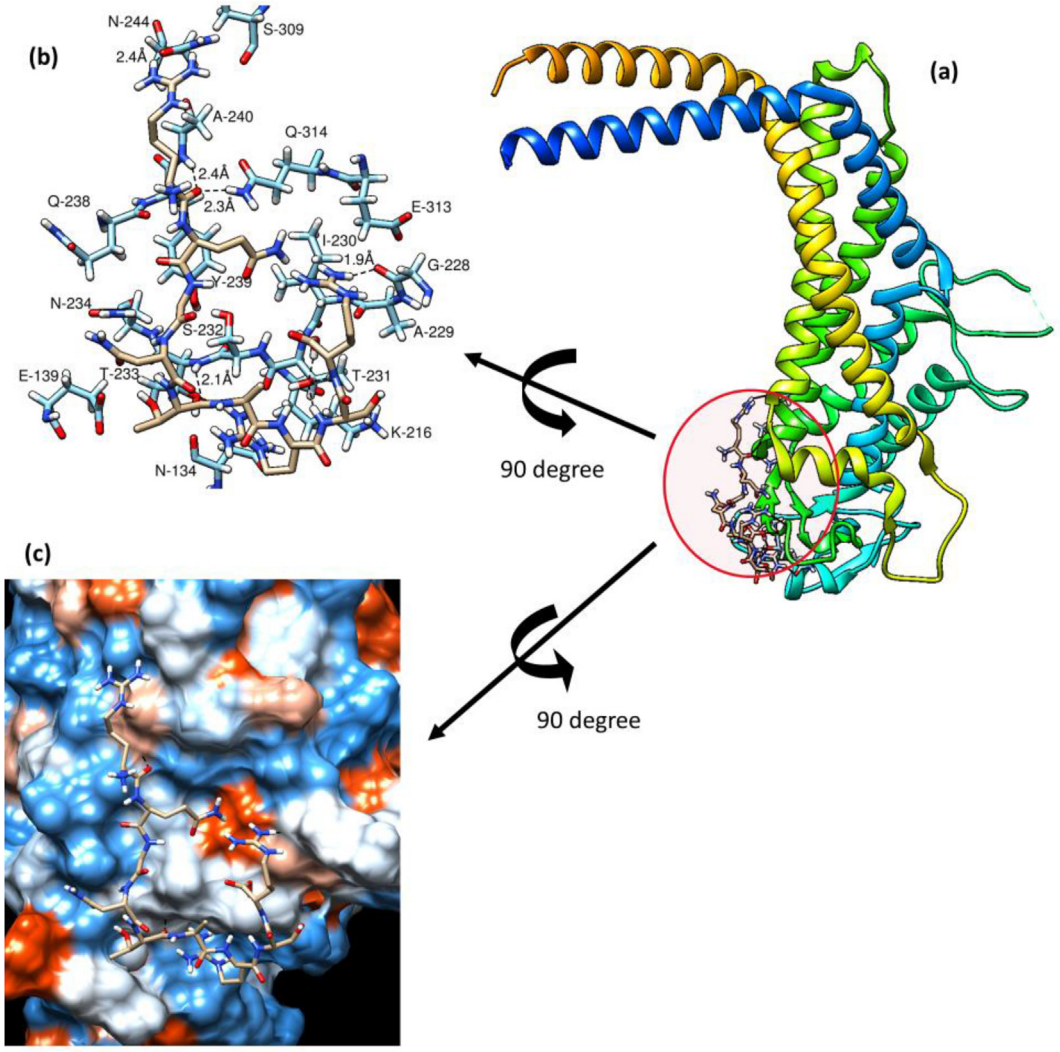

Figure 4. (a) Binding of pepsin-resistant wheat germ peptides P262 at the allosteric site of *H. pylori* adhesin SabA (b) interacting amino acids within 3Å and hydrogen bond pattern (c) charge environment and hydrophobicity/hydrophilicity of the binding pocket in the Kyte-Doolittle scale with colors ranging from dodger blue for the most hydrophilic to white 0.0 to orange red for the most hydrophobic.

binding affinity (kcal/mol) was calculated based on the sum of binding forces such as electrostatic interactions, van der Waals force, hydrogen bonding, entropy and conformational state of ligands (Pantsar and Poso, 2018). The binding affinities of sixteen peptides to BabA and SabA range from -6.0 to -7.4 and -6.0 to -7.8 kcal/mol, respectively. These values indicate medium to strong binding of the peptides to the adhesins, with the larger magnitude negative numbers implying that a relatively low concentration of the peptide is adequate to maximally occupy a ligand-binding site on the adhesin proteins and trigger a physiological response. Also, higher negative value of the binding affinity indicates spontaneous formation of a more stable complex. Previous studies have shown that BabA binding to the Lewis^b antigens does not cause any conformational changes in the adhesin protein (Hage et al., 2015). Therefore, it is imperative to screen bioactive peptides that could bind the active site of BabA at high specificity and affinity, as allosteric binding might not cause any conformational change in the protein that could inhibit its binding with gastric mucosa antigens. Hence, the site of peptide interaction is crucial to inhibit the interaction of adhesin proteins with the Lewis antigens in the gastric mucosa.

The binding site of BabA is situated within the β-strand motif and binding is mainly facilitated by networks of hydrogen bonding between two fucose residues (Fuc1 and Fuc4), one galactose residue Gal5) and an N-acetylglucosamine residue (GlcNAc3) of the hexasaccharide form of the Lewis^b antigen and a total of eight amino acid residues of BabA (C-189, G-191, N-194, N-206, D-233, S-234, S-244 and T-246) (Hage et al., 2015). The crystal structure of soluble extracellular adhesin domain of SabA (PDB 4O5J) was used in this study and the binding site is within the vicinity of S-80, P-81, W-97, Y-148, K-152, Q-159 and Q-162. In Table 2, our study showed that in both BabA and SabA, 14 peptides bound in the vicinity of the active site of the adhesin proteins whereas P70 and P193 in BabA and P184 and P262 in SabA demonstrated allosteric binding. Some of the 14 peptides (P2, P86, P207, P210, P249; and P2, P70, P73, P115, P193, P210) bound in the region of the active site of BabA and SabA, respectively, with appreciable affinity and hydrogen bonding network to form more stable complex. Hence, these peptides would be more effective at preventing *H. pylori* infection in the gastric wall as the active site of the adhesin proteins would be largely pre-occupied with peptides.

It has been reported previously to bind selectively to sialyl-Lewis^x and Lewis^x antigens but not to Lewis^b, Lewis^b or Lewis^y (Pang et al., 2014). There was no strong correlation between the strength/stability of the binding at the vicinity of the active site and allosteric site. The various peptides binding at the allosteric site of BabA (P70 and P193) and SabA (P184 and P262) showed relatively low affinity and stability. However, while peptides binding in the vicinity of the active site of BabA (P2, P86, P207, P249) and SabA (P2, P70, P73, P115, P193) showed strong binding affinity and stability, other peptides such as P89, P102, P115, P213, P264

Table 3. *In silico* absorption, distribution, metabolism, excretion and toxicity (ADME/Tox profile of the selected 16 anti-adhesive peptides generated using SwissADME and ToxinPred

		Physicochemical properties					Toxicity	Lipophilicity		Drug-likeness		Pharmacokinetics		
No.	Sequence	MW (g/mol)	ROTB (n)	HBA (n)	HBD (n)	ESOL Log S	Toxin [SVM score]	TPSA (Å^2)	CLogP o/w	Bio-availability score	Lipinski filter	GIA	P-glycoprotein substrate	CYP34A inhibitor
2	DAVTYTEHAR	1162.21	45	21	20	0.08 HS	-1.14 No	551.09	-4.02	0.17	No	Low	Yes	No
70	ISANIAAR	814.93	34	13	14	0.83 HS	-1.23 No	392.24	-3.07	0.17	No	Low	Yes	No
73	PAGNVGEIR	912.00	38	15	15	0.64 HS	-1.20 No	424.42	-3.64	0.17	No	Low	Yes	No
86	TIVQQVEAYR	1206.35	49	19	19	-0.94 VS	-1.16 No	551.06	-2.52	0.17	No	Low	Yes	No
89	IISSIEQK	917.05	40	16	14	1.59 HS	-0.78 No	413.89	-2.46	0.17	No	Low	Yes	No
102	DNIQGITK	887.98	38	16	14	2.96 HS	-1.78 No	436.75	-3.67	0.17	No	Low	Yes	No
115	MISVTGPR	860.03	34	13	13	-0.14 VS	-1.05 No	385.89	-2.07	0.17	No	Low	Yes	No
184	VPIPNPSGDR	1051.15	38	17	14	0.40 HS	- 0.91 No	461.37	-4.34	0.17	No	Low	Yes	No
193	RVTIMPK	884.08	34	12	12	-0.96 VS	-1.29 No	362.58	-1.33	0.17	No	Low	Yes	No
207	AVVIHVPYR	1053.26	38	14	14	-3.76 S	-1.22 No	398.14	0.18	0.17	No	Low	Yes	No
210	VTGAIPI	669.81	24	10	8	-1.09 VS	-0.63 No	249.36	-0.31	0.17	No	Low	No	No
213	KMEVPYCIVK	1209.52	48	17	14	-2.21 S	-0.20 No	490.10	0.47	0.17	No	Low	Yes	No
220	KAVVIHVPYR	1181.43	45	16	16	-3.78 S	-1.25 No	453.26	0.07	0.17	No	Low	Yes	No
249	MDNNTVGGSR	1050.10	45	19	19	3.47 HS	-1.02 No	576.36	-6.68	0.17	No	Low	Yes	No
262	RQGNTARSR	1045.11	47	18	23	4.49 HS	-1.03 No	608.46	-7.60	0.17	No	Low	Yes	No
264	VTMVEIE	819.96	33	14	11	-1.32 VS	-0.66 No	358.05	-0.45	0.11	No	Low	No	No
	Rebamipide (Negative control)	370.79	6	4	3	-3.70 S	N/A	99.26	2.70	0.56	Yes	High	No	No
	3-siallyllactose (Positive control)	633.55	16	19	13	2. 01 HS	N/A No	342.92	-5.76	0.11	No	Low	Yes	No

Abbreviations: molecular weight (g/mol) (MW); number of rotatable bonds (**ROTB**); hydrogen bond donors (**HBD**); hydrogen bond acceptors (**HBA**); estimated solubility (**ESOL**) with solubility classes (HS - highly soluble, VS - very soluble, MS - moderately soluble, S - soluble); topological polar surface area (**TPSA**); logarithm of compound partition coefficient between *n*-octanol and water (**CLogP**); Lipinski filter (Lipinski's rule-of-5); gastrointestinal absorption (**GIA**), P-glycoprotein substrate and CYP3A4 inhibition.

for BabA and P102, P213, P220, P249 for SabA, that equally bound in the vicinity of the active site, demonstrated relatively low affinity and stability. Therefore, the site of the interaction could not be completely attributed for the various energy differences.

Interestingly, P86 demonstrated the highest affinity for BabA, which could be due to favourable electrostatic, Van der Waals and hydrophobic interactions within the active site of the protein as well as well-ordered 11 hydrogen bonding patterns in BabA–P86 complex involving the residues K154, K155, N196, V201, K214, I215, T217, D219 and Q265 (Table 2, Figure 1a, b & c) within a bond distance of 2.0–2.7 Å. Although hydrogen bonding played a role in SabA binding to glycerol involving K152 and Q159 of the adhesin protein (Pang et al., 2014), the highest binding affinity of P210 to SabA could be mainly contributions from other forces rather than only 3 hydrogen bonding patterns in SabA–P210 complex involving the residues K152, T333 and Q338 at bond distance between 2.1–2.4 Å (Table 2, Figure 3a, b, & c). P210, which demonstrated strong affinity for BabA (7.0 kcal/mol) and SabA (7.7 kcal/mol), formed only 4 and 3 hydrogen bonding with BabA and SabA, respectively. Yet the peptide had strong affinity for the adhesin proteins compared to other peptides with higher number of hydrogen bonding network (Table 2). The strong binding potential of P210 to both adhesin proteins could be attributed to strong hydrophobic interaction. Figure 3b & c showed that P210 interacted appreciably with hydrophobic amino acids and was almost completely engulfed into the hydrophobic core of the protein as this peptide is the most hydrophobic among the reported peptides (Sun et al., 2020a). Overall, P86 had the strongest affinity for BabA but did not show the same effect on SabA.

Likewise, P193 had the closest affinity for SabA as P210 but it bound only in the allosteric site of BabA. Therefore, P210 having shown great binding affinity for both adhesin proteins in their various active sites, is predicted as the most promising peptide in the database for further development as a nutraceutical against *H. pylori* infection. It is also noted that binding affinity alone does not determine potency. Potency is a complex interplay of both binding affinity and ligand efficacy, which is the ability of the ligand to produce biological response upon binding to the target receptor (Kenakin, 2006). As such, in order to further select best candidates for validation, more studies will need to look into ligand efficacy as well as to incorporate information of aliphatic and instability

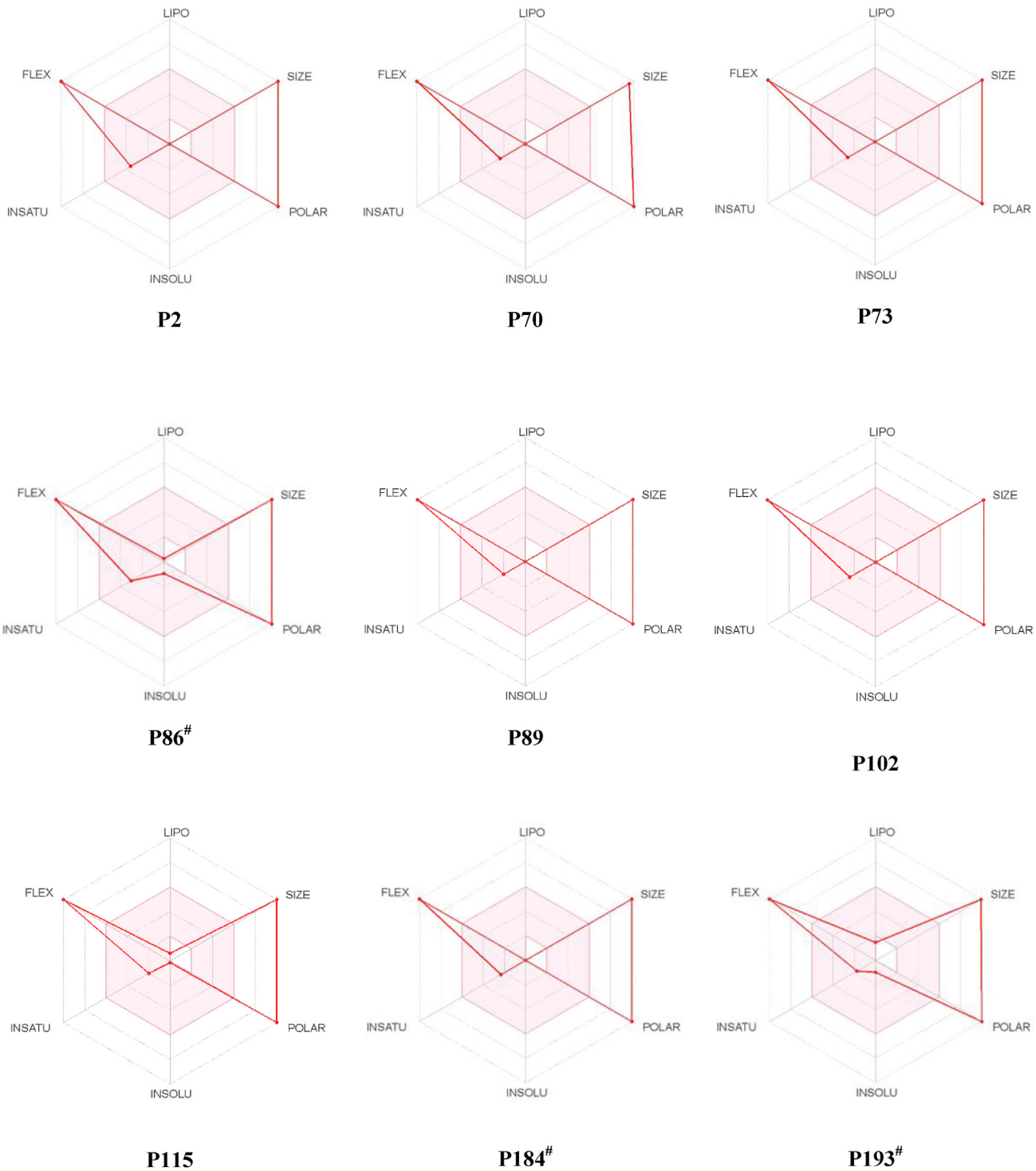

Figure 5. Bioavailability radar images of the anti-adhesive peptides, rebamipide (negative control) and 3-sialyllactose (positive control) generated using SwissADME. Bioavailability radar displayed six physiochemical properties: lipophilicity, size, polarity, solubility, flexibility and saturation. #Selected as representative anti-adhesive peptides.

indexes of the peptides, which predict peptide stability in lab benchwork for food thermal processing (Sun et al., 2020a).

3.3. *In silico physicochemical properties and drug-likeness*

The drug-likeness and medicinal chemistry friendliness of the sixteen peptides with binding activities to *H. pylori* adhesins were predicted and evaluated based on physiochemical and pharmacokinetic properties using SwissADME (Table 3). The pharmacokinetic properties were estimated based on ADME parameters. In this study, 3-sialyllactose sodium salt from human and bovine milk that has been clinically studied extensively as an anti-adhesive agent against *H. pylori* infection was selected as a positive control. Rebamipide, a gastroprotective drug that interferes with *H. pylori* adhesion by acting on gastric epithelial cells rather than *H. pylori*, was chosen as a negative control (Suzuki et al., 1994).

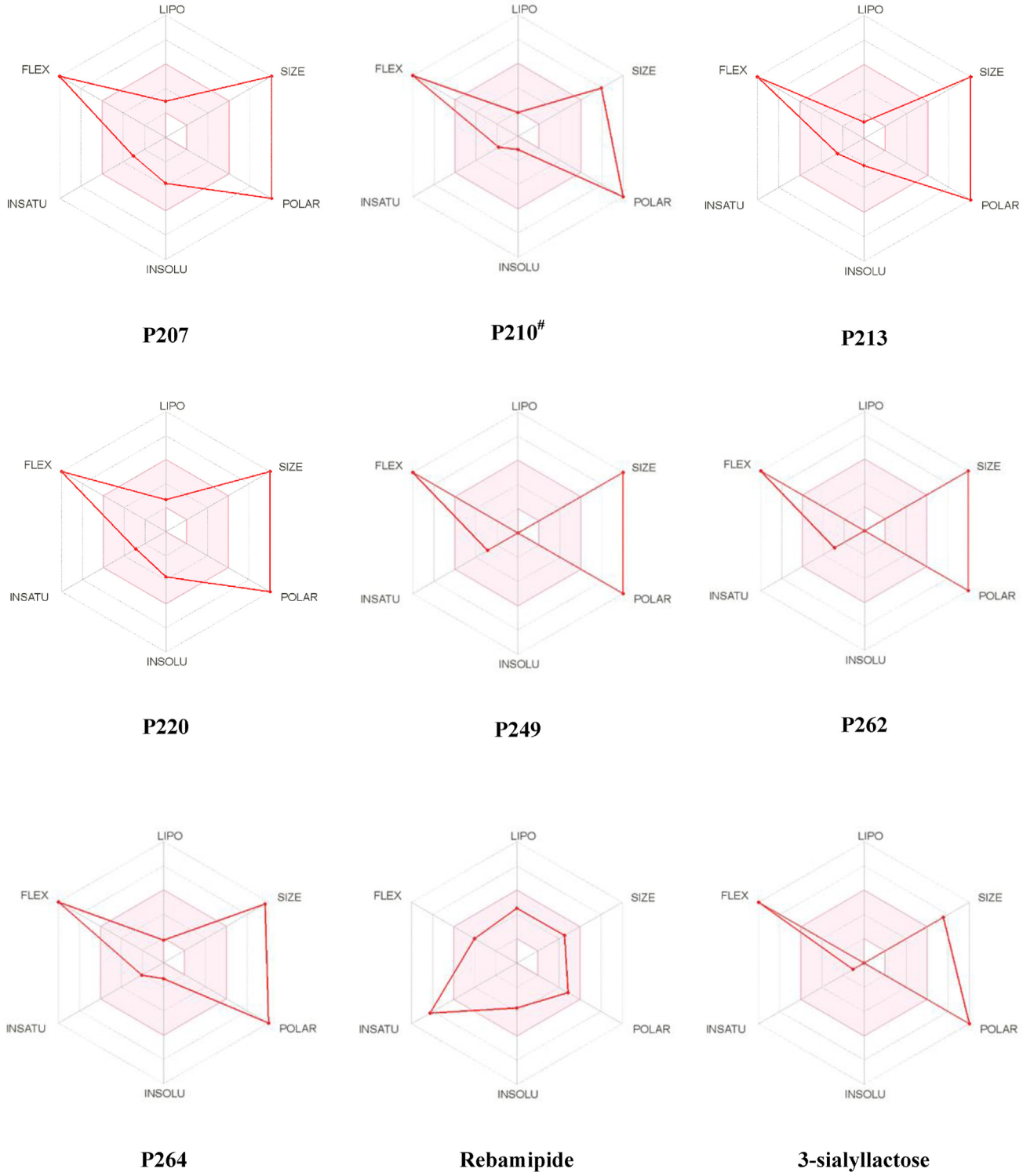

Figure 5. (*continued*).

As shown in Table 3, all peptides had relatively high molecular weight (ranging from 669.81 to 1209.52 g/mol). Additionally, oral bioavailability of a compound could be predicted based on two properties including flexibility (ROTB - the number of rotatable bonds –) and polarity (TPSA - topological polar surface area) (Ji et al., 2020). Compounds with >10 rotatable bonds have been associated with poor oral bioavailability (equated to high molecular weight and structure rigidity), while compounds with low topological polar surface area (between 20 and 130 $Å^2$) tend to have high oral bioavailability (Veber et al., 2002; Mbarik et al., 2019). According to Table 3, all sixteen peptides showed values of TPSA >140 $Å^2$ and ROTB >10, indicating that they have poor oral bioavailability. The bioavailability scores of the peptides were also low (0.11 & 0.17), similar to 3-sialyllactose (0.11) and much lower than Rebamipide (0.56), in compliance with predicted oral bioavailability.

Bioavailability radar images of the peptides were shown in Figure 5. Bioavailability radar represents a rapid appraisal of the drug-likeness of a

molecule by six physiochemical properties: lipophilicity, size, polarity, solubility, flexibility and saturation (Daina et al., 2017). The closer the parameter to the centre of radar, the smaller the value. The selected peptides generated very similar bioavailability radar images, specifically in size, polarity and flexibility parameters. Four peptides (P207 - AVVIHVPYR, P210 - VTGAIPI, P213 - KMEVPYCIVK and P220 - KAVVIHVPYR) showed higher lipophilicity, as they were also shown to be hydrophobic in the primary database (Sun et al., 2020a; van de Waterbeemd et al., 1994). The radar images also resembled that of 3-sialyllactose (in flexibility and polarity parameters), considering the similarities in physiochemical properties to the peptides, and different from that of Rebamipide (higher lipophilicity, smaller size, and much higher saturation).

All sixteen DWPGH-derived bioactive peptides were shown to be non-toxic, with negative SVM (support vector machines) scores, ranging from -0.07 to -2.10. They also did not pass the Lipinski filter, which is based on Lipinski rule-of-five, a rule of thumb to evaluate drug-likeness of a compound to be orally active in humans (Ji et al., 2020). The peptides all violated at least three rules, due to high molecular weight (>500 g/mol), having more than 10 hydrogen bond acceptors (HBA) and more than 5 hydrogen bond donors (HBD). Moreover, aqueous solubility (EStimated SOLubility - ESOL) is one of the key chemical physical properties of interest that affects the uptake and distribution of biologically active compounds, thus affecting their potential efficacy (Delaney, 2004). Among the selected peptides, eight were highly soluble, five were very soluble, and three were soluble. Lipophilicity is measured by the partition coefficient between *n*-octanol and water ($ClogP_{o/w}$). Lipophilicity is related to the permeability of a compound through biological membranes (Durá n-Iturbide et al., 2020) and is also considered as an indicator for toxicity in short-term animal studies (Zafar et al., 2020). All of the peptides have ClogP values < 3, thus they could diffuse passively through biological membranes and are not toxic.

SwissADME adapted the Brain Or Intestinal EstimateD permeation (BOILED-Egg) model that predicts GIA and blood-brain barrier permeation using lipophilicity and polarity of molecules (Daina and Zoete, 2016). Prediction of passive gastrointestinal absorption (GIA) was low for the sixteen peptides. Another important contribution to drug absorption is P-glycoprotein substrate. P-glycoprotein (permeability glycoprotein, or multidrug resistance protein 1) is a membrane transport protein that functions as an efflux transporter for xenobiotic compounds (including drugs). P-glycoprotein is therefore responsible for limiting the accumulation of cytosolic drugs (Ji et al., 2020). All sixteen peptides were good substrates to P-glycoprotein, and would be exported out of intestinal cells by transporter enzymes. This suggested that these bioactive peptides would have low intestinal absorption and bioavailability, which are desirable properties for the anti-adhesive activity. Anti-adhesive peptides need to maintain intact structure in the gastrointestinal tract to target and interact with *H. pylori*, and being readily absorbed by gastric cells would hinder this preventive action.

Lastly, CYP3A4 is one of the most common enzyme subtypes involved in drug metabolism, and a member of the drug-metabolizing cytochrome P450 family whose role has been well-established (Zanger and Schwab, 2013). Induction of a compound with any of CYP isoenzymes could lead to fast metabolism of drugs that may result in overdose. Meanwhile, if CYP isoenzyme(s) inhibit a compound, it could lead to toxicity due to the accumulation of drug in the system. Both scenarios are undesirable. The DWGPH-derived peptides were predicted to not inhibit CYP3A4 isoenzyme, therefore would not likely accumulate and lead to toxicity. This property is desirable considering the targeted application of the food-derived peptides as anti-adhesive nutraceutical candidates.

4. Conclusion

This study confirmed the interactions between DWPGH-derived anti-adhesive peptides and the two dominant *H. pylori* adhesins, BabA and SabA, using molecular docking simulation. The peptides occupy the binding pocket of BabA and SabA, which is possibly responsible for their anti-adhesive activity against *H. pylori* since binding at allosteric site does not cause conformational changes in the protein. This work provided new insights into the anti-adhesive mechanism for continuous discovery and identification of readily available, cost-effective, and highly potent anti-adhesive agents in the future. Moreover, 33 out of 267 DWPGH-derived peptides were biostable in the gastric digestion phase *in silico*. The ADME/Tox profile indicated that these peptides have no toxicity, low bioavailability and poor intestinal absorption. Taken together, the findings demonstrate that the wheat germ peptides, especially P210 (VTGAIPI), have strong potential as nutraceutical candidates for preventing *H. pylori* infection.

Declarations

Author contribution statement

Chi Dang, Ogadimma Okagu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiaohong Sun: Conceived and designed the experiments; Analyzed and interpreted the data.

Chibuike C. Udenigwe: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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