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A comprehensive review on the pancreatic lipase inhibitory peptides: A future anti-obesity strategy

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ABSTRACT

Dysregulation of lipid homeostasis contributes to obesity and can directly lead to several critical public health concerns globally. This paper aimed to present a brief review of related properties and the use of pancreatic lipase inhibitors as the future weight loss drug discovery and development procured from a wide range of natural sources. A total of 176 pancreatic lipase inhibitory peptides were identified from recent publications and peptide databases. These peptides were classified into three categories according to their peptide length and further analyzed using bioinformatic approaches to identify their structural activity relationship. Molecular docking analyses were conducted for each amino acid at the terminal position of the peptides to predict the binding affinity between peptide-enzyme protein complexes based on intermolecular contact interactions. Overall, the observations revealed the features of the inhibitory peptides and their inhibitory mechanisms and interactions. These findings strived to benefit scientists whose research may be relevant to anti-obesity drug development and/or discovery thereby support effective translation of preclinical research for humans' health being.

Keywords: anti-obesity, drug development, inhibitory peptides, molecular docking, pancreatic lipase, structural activity relationship

INTRODUCTION

Obesity is a complex disease involving an excessive and abnormal accumulation of fat in all areas of the body and particularly the abdominal region. According to the World Health Organization (WHO) consultation, individuals with a body mass index greater than or equal to 25 are considered overweight; and body mass index greater than or equal to 30 are considered as obese. As of June 2021, more than 1.9 billion adults, 18 years and older, were overweight; of these over 650 million were obese. This issue has grown to epidemic proportions with over four million people dying each year as a result of being overweight or obese, according to the global burden of disease [1]. Importantly, obesity impact on society in different ways and contributes to the socioeconomic and health care burden due to associated comorbidities. Obesity has led to various medical comorbidities that may increase the risk of other diseases including stroke, cardiovascular diseases, sleep apnea, osteoarthritis, dyslipidemia, non-alcoholic fatty liver disease, type II diabetes, hypertension, chronic renal disease, infertility and some forms of cancer [2, 3]. These comorbidities also give rise to a momentous psychosocial burden, impacting on numerous areas of psychosocial functioning that leads to poorer mental health outcomes and reduced quality of life as people living with obesity face substantial bias and stigma [4]. Hence, prevention of obesity will consequentially lead to a reduction in mortality and the prevalence of chronic metabolic illnesses.

At present, the commonly available therapeutic strategy for overweight and obese individuals in modern society include weight management programs, special diets i.e., calorie restriction and intermittent fasting, weight loss devices, weight loss medicines, bariatric surgery and liposuction [5]. However, certain weight loss strategies, such as bariatric surgery or liposuction, expose patients to surgical risks and in some cases death and are concerns that have been frequently reported [6]. Although the present use of non-prescription dietary supplements and safer short-term treatments coupled with long term diet plus exercise may attain weight loss, other numerous weight loss medications have been withdrawn from the market due to serious adverse effects. For instance, the use of aminorex and sibutramine have been reported to cause cardiovascular toxicity, pulmonary hypertension and some excess non-fatal cardiovascular events [6]. Therefore, prevention of obesity by means of balanced diets will be essential to reduce the prevalence and mortality of chronic metabolic diseases. New treatments for obesity that are both

more efficacious and better tolerated are urgently needed. Moreover, the ideal anti-obesity drug should produce sustained weight loss with minimal side effects.

This paper aimed to explore the potential of peptides which inhibit pancreatic lipase obtained from the natural world as an anti-obesity therapeutic approach. For instance, pancreatic lipase inhibitory peptides have been obtained from plant-based sources i.e., millet grain [7], rice bran [8], oat bran [9], cocoa protein hydrolysates [10], cumin seeds [11], spent brewer's grain [12], hazelnuts [13], soybeans [14], fermented beans [15], and onions [16]. On the other hand, pancreatic lipase inhibitory peptides have been derived from marine-based sources such as sea cucumbers [17], tuna fish [18], shark fish [19], miiuy croaker fish [20], and dried seaweed-spirulina [21]. Moreover, insect-based sources have been derived from the tropical house cricket, mealworms and desert locusts [22]. In addition to that, pancreatic lipase inhibitory peptides have been acquired from dairy products for example, cow casein [23] and camel casein [24, 25].

Over the last half decade, there have been plenty of studies focussing on isolating multifunctional peptides from food and food waste with bioactivity potential in anti-obesity. Some peptides are isolated and identified from proteins, and others have been synthesized to investigate their properties. Enzyme produced by these peptides contribute to the digestion of carbohydrate and fat, and their inhibition activity could scale down the absorption of these macronutrients and make them an excellent target for designing anti-obesity compounds. For instances, oat bran-derived peptides were used in food formulations due to their multifunctional anti-obesity properties [8, 9]. Synthetic lipase inhibitory peptides derived from millet grains were also identified as the potent biopeptides associated with metabolic syndrome [7]. In addition to that, techniques for isolation and identification of pancreatic lipase inhibitory peptides were studied using high performance liquid chromatography (RP-HPLC) and sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE). Peptide identification was also carried out using liquid chromatography mass spectrometry-linear trap quadropole (LCMS-LTQ) orbitrap and gas chromatography-mass spectrometry (GC-MS) approach from various protein hydrolysate, i.e., brewer's spent grain, hazelnuts, *spirulina platensis*, edible insects and camel milk [12, 13, 21-25]. Likewise, in-silico evaluations of potential drug-like inhibitors of pancreatic lipase involved the SwissADME prediction tool, semi-empirical quantum mechanics (SQM), molecular electrostatic potential (MEP) and molecular docking analysis. Their chemical reactivity and detailed molecular interactions of identified compounds with pancreatic lipase at its catalytic site domain were also elucidated and discussed [10, 18]. Collectively, these reports illustrate few of the remarkably origins for peptide drug discovery and development.

To better interpret the amino acid preference of peptide inhibitors, a total of 176 pancreatic lipase inhibitory peptides were examined (**Table A1** in **Appendix A**). The peptides were divided into three groups based on peptide length i.e., short peptides (two-five amino acid residues), medium peptides (six-10 amino acid residues) and long peptides (>10 amino acid residues). Analysis of the hotspots of pancreatic lipase inhibitory peptides and reactive amino acid from the selected inhibitory peptides were accessed using the Pepsite2 server (http://pepsite2.russelllab.org/, accessed on 7/5/2022) with lipase (PDB ID: 1ETH) as the target protein [11] (**Table B1** in **Appendix B**). The properties of the selected peptides were classified for instance by the hydrophobicity-aliphatic or aromatic side chains; hydrophilicity-acidic or basic; and polar neutral which contribute to the binding interactions with pancreatic lipase active sites. Furthermore, the preference for active amino acids at both N- and C-terminal positions for each peptide were studied, as some amino acids at these positions may interact actively with the pancreatic lipase enzyme, thus exerting inhibitory effects. The predicting peptide structures from amino acid sequences were analyzed using the Pep-fold3 server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/, accessed on 18/5/2022) [26] and the sequences were submitted to HADDOCK 2.4 @Bonvinlab (https://wenmr.science.uu.nl/haddock2.4/submit/1, accessed on 19/5/2022) [27]. The prediction of the binding affinity between peptides-enzyme protein complexes was based on intermolecular contacts interaction (e.g.: hydrophobic interaction, salt bridge, and hydrogen bond) and was further analyzed via the protein binding energy prediction (PRODIGY) web application (https://wenmr.science.uu.nl/prodigy/run/lxi_3LyHSjDO, accessed on 28/5/2022) [28]. Lastly, the schematic illustration of peptides-enzyme interactions were plotted with LigPlot$^+$ version 2.2.5 software (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/, accessed on 23/6/2022) [29] and results were tabulated in **Supplementary Materials 1**. All the findings are further illustrated in the following sections.

A BETTER UNDERSTANDING OF HUMAN PANCREATIC LIPASE

The human pancreatic lipase (EC 3.1.1.3) is made up of 449 amino acids with an estimated protein molecular weight of 49,558 Da and a single N-terminal linked glycosylation site found at ASN140 with its tertiary structure determined by radiographic crystallography at 2.8 Å. The human pancreatic lipase enzyme can be categorized into two domains: a larger globular N-terminal domain formed by a central β-sheet core extending from amino acids one to 336; and a c-terminal domain extending from amino acids 337 to 449 comprised of a β-sheet sandwich structure modelled by two layers of β-sheet with each consisting of four antiparallel strands. These domains are separated by a short, unstructured stretch of amino acids which are stabilized by seven disulphide bonds [30, 31]. N-terminal domain residues bears the catalytic triad, which is buried underneath a surface loop controlling the access of substrate, whereas the C-terminal domain is mainly devoted to colipase binding [32]. The crystal structure of human pancreatic lipase requires a catalytic triad similar to the serine proteases, where the SER153, ASP177, and HIS264 comprise the triad with SER153 and is located near to HIS264 and ASP177 [33]. In the crystal structure, SER153 is located in the N-terminal domain with its side chain linked to HIS264, which in turn is linked to ASP177 by hydrogen bonds in a topology that mimics the catalytic triad of trypsin [3, 34]. This catalytic triad contains the active site analogous to that present in serine proteases and is covered by several surface loops, with the position of these loops sterically hindering the access of substrate to the catalytic sites. The largest loops of this crystal formed the lid domain, linked by a disulphide bridge between CYS238 and CYS262. The remainder of the two shorter loops, formed by (β-5 fold) residues 76-80 and residues 213-217, impede the catalytic site. Thus, before lipolysis can proceed, these loops must alter the crystal structure

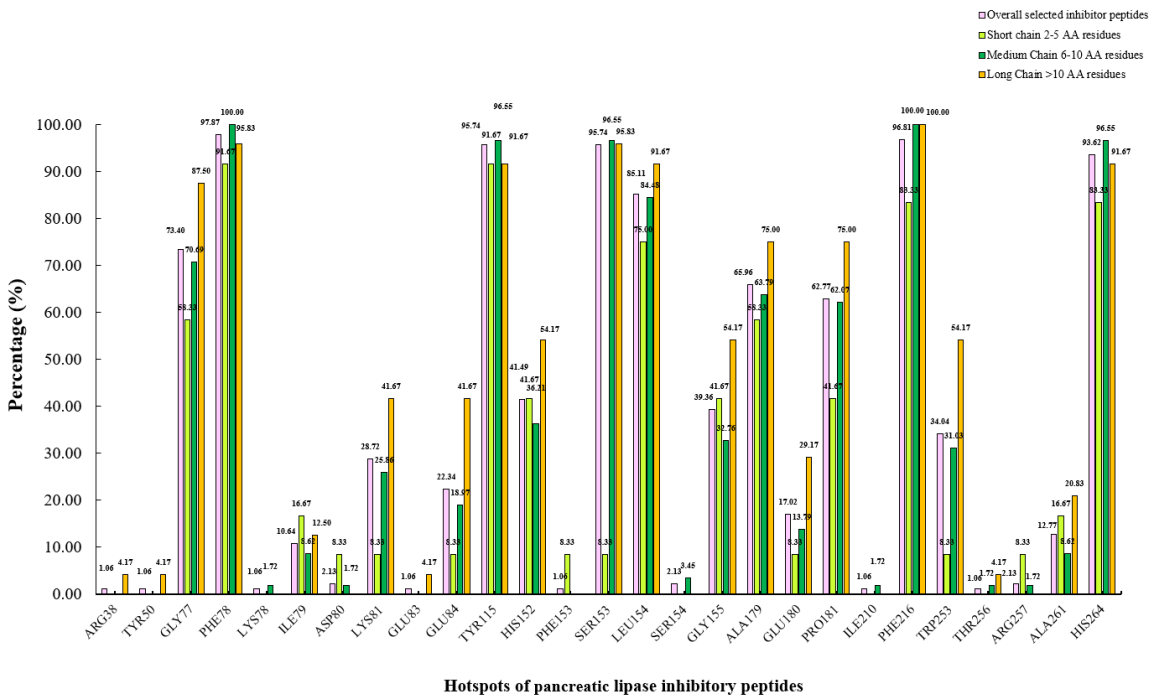

Figure 1. Binding frequency of overall bound hotspots & bound hotspots of pancreatic lipase inhibitory peptide on peptide length (Source: Authors' own elaboration)

conformation and open the active site to substrate. As a consequence, this reconfiguration makes this part of the pancreatic lipase enzyme obsolete and determines the decomposition capacity to control the free fatty acids from entering the blood to achieve lipid lowering actions [3, 34]. Also, the inhibitory effect could be a consequence of the interaction of the inhibitory peptides with PHE78, HIS152, and PHE216 amino acid residues of the pancreatic lipase enzyme which may hinder the substrate binding in view of the fact that this site is an oxyanion hole that holds the substrate at the active site and destabilizes the transition state intermediates preventing the enzyme from hydrolyzing lipids and ultimately curtailing the lipid absorption [35].

Efficient absorption of dietary fats is crucial for life and good health and is dependent on the reciprocated action of lipase. There are three lipases found in higher vertebrates: i.e., pancreatic, hepatic and gastric/lingual that are structurally closely related to each other and hydrolyze triglycerides in chylomicrons and very low-density lipoproteins. Among them, pancreatic lipase, also known as pancreatic triacylglycerol lipase or steapsin, plays a pivotal role in dietary fat absorption by hydrolyzing dietary long chain ester linkage triacylglycerols at positions one and three, producing mainly two-monoacylglycerol and free fatty acids [36]. In human intestine, the optimal activity of this enzyme is reached when a protein co-enzyme, called colipase, is present in the duodenum along with bile acids. The lipid mixed particles are absorbed by the small intestine followed by re-synthesis of triacylglycerol energy stores in adipose tissues [3]. However, rapid digestion of dietary fat by lipases exaggerates the accumulation of free fatty acids, cholesterol and lipoprotein within blood which may lead to the occurrence of metabolic syndrome related comorbidities such as cardiovascular diseases, insulin resistance, type 2 diabetes and non-alcoholic fatty liver diseases [37]. Therefore, research approaches aimed at inhibiting pancreatic lipase enzyme could play a critical role in controlling the level of free fatty acids accumulation in bariatric patient by delaying and blocking dietary fat absorption [3].

THE PREFERENCE OF THE LIPASE HOTSPOTS AND THEIR POSSIBLE INHIBITORY MECHANISMS

Since first being developed in the 1980s, molecular docking has represented a unique in silico tool to assist drug design and discovery. Advancements in the experimental screening of large libraries of compounds against panels of molecular targets, has shed light for discovering biologically active hits. However, the high costs required to establish and maintain these screening platforms often hamper their use for drug discovery. Nevertheless, molecular docking analysis enables the identification of novel compounds, predicting ligand-target interactions or delineating structure-activity relationships of therapeutic interest compounds at a molecular level without knowing the priori chemical structure of other target modulators. In this review, we illustrated on how molecular docking was able to assist in drug discovery tasks via the peptide-enzyme interactions.

The biological functions of the protein interaction networks are complex. This paper presents an overview of the findings in current research focusing on protein interactions correspond to peptide-mediated interactions predicted with the PepSite 2 tool. It relies on the preferred peptide-binding environments which were calculated from a set of known protein-peptide three dimensional structures, linked with distance constraints deduced from known peptides. Based on the results, it was found that 27 hotspots were present in pancreatic lipase inhibitor peptides. To expand these observations, the binding frequency of the overall bound hotspots and individual bound hotspots of pancreatic lipase inhibitor peptides have been studied extensively according to their peptide lengths which correspond to their active characteristic during the catalytic process (**Figure 1**).

The results obtained from the overall structure activity relationship analysis of the selected inhibitor peptides showed

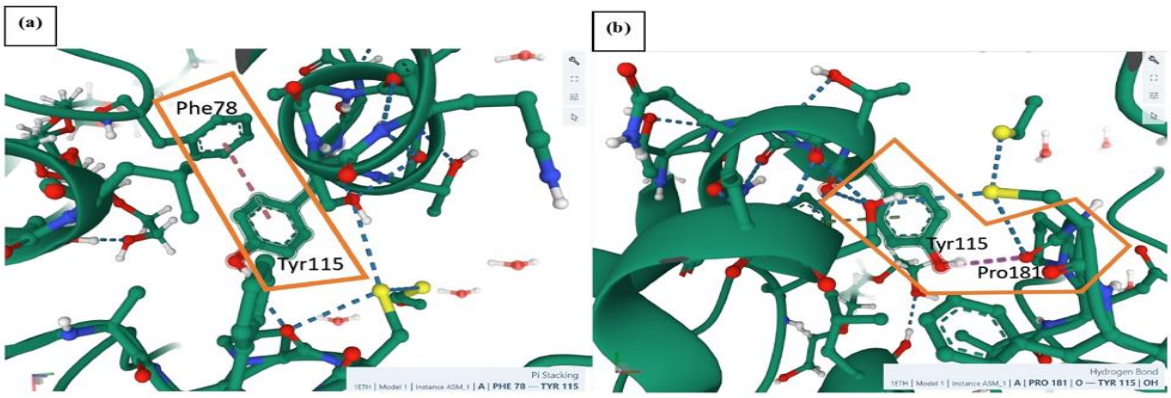

Figure 2. Three-dimensional diagrams of (a) pi-stacking interaction between Tyr115 & Phe78 as well as (b) hydrogen bond formed between Tyr115 & Pro181 in lipase (1ETH) on RSCB protein data bank (https://www.rcsb.org/3d-view/1ETH)

that PHE78 (98%), TYR115 (96%), HIS264 (94%), LEU154 (85%), GLY77 (73%), ALA179 (66%), and PRO181 (63%) tend to be bound along with the catalytic residues, such as SER153 (95%) and PHE216 (96%). In details, the short chain inhibitory peptides that contain 2-5 amino acid residues have a tendency to bind to PHE78 (91%), TYR115 (91%), PHE216 (83%), HIS264 (83%), LEU154 (75%), GLY77 (58%), and ALA179 (58%); the medium chain inhibitory peptides that contain 6-10 amino acid residues were likely bound to PHE78 (100%), PHE216 (100%), TYR115 (97%), SER153 (97%), HIS264 (97%), GLY77 (71%), ALA179 (64%), and PRO181 (62%); the long inhibitory peptides that contain >10 amino acid residues have the high binding frequency residues in the following order: PHE216 (100%), PHE78 (96%), SER153 (96%), TYR115 (92%), LEU154 (92%), HIS264 (92%), GLY77 (88%), ALA179, (75%), PRO181 (75%), GLY155(54%), HIS152 (54%), and TRP253 (54%).

As aforementioned, this pancreatic lipase enzyme contains the catalytic triad of SER153, ASP177, and HIS264. In particular, SER153 is an essential element of the catalytic site in human pancreatic lipase. An X-ray diffraction analysis of human pancreatic lipase concluded that SER153 was the nucleophilic residue required for catalysis reaction [33]. Two residues of the catalytic triad (i.e., SER153 and HIS264) were totally blocked by the peptides regardless of their length; however, ASP177 was, surprisingly, not involved. Nevertheless, ALA179 and PRO181, which are located near ASP177, was highly bound by these peptides. Other residues that located close to the triad such as LEU154 and GLY155 (near to SER153), were bound, albeit to a lesser extent. Thus, this direct or indirect binding of catalytic triad may determine degree of inhibition of lipase activity. The interaction of inhibitory peptides with the substrate-binding residues, such as PHE78, ILE79, HIS152, PHE216, TRP253, and ARG257 [24], may be only one aspect function manipulation. Indeed, it was suggested that chemical modification of the SER152, which was found in porcine lipase that constitutes the ternary active core of the C-terminal edge positioned in the N-terminal domain proximal to the β-layer of the double helix structure, significantly reduced its activity [38]. Based on the present observations, PHE78 and PHE216 residues tend to be bound by all peptides whereas the HIS152 residue have a propensity to interact with long inhibitory peptides. By having these peptides around the substrate binding sites, lipid (substrate) will have a lower accessibility to the lipase as a result of the impediment or the steric hindrance effects on intramolecular interactions.

Aside from this, the opened lid domain (residues 76-80 and 213-217) that is formed by the loop buried in the active site also enhances the binding capacity of the substrate and lipase. Therefore, the substrate can penetrate easily into the hydrophobic channel and subsequently be bound to the active site [3, 33]. Therefore, the present data suggested that the GLY77 residue was highly bound to the peptides. This interaction prevented the enzyme from the opening of the lid by reducing its flexibility on the loop and hence the enzyme remained in its quiescent form, which eventually lead to the enzyme unable to unmask the active site or turn into a partially active form.

It should also be highlighted that TYR115 is one of the preferred hotspots. Hitherto, no reports have ever been disclosed about the importance of this residue. When the 3D structure of the lipase (**Figure 2**) was examined, it was found that this residue actually bound to PHE78 (substrate binding site) via pi-stacking, and with PRO181 via hydrogen bond, which is close to the catalytic triad, through a hydrogen bond. It was hence hypothesized that binding to TYR115 could possibly provide steric hindrance to the active sites or alter the enzyme conformation. Overall, the inhibitory activities of pancreatic lipase could be ascribed by the absence of hotspots or partial modification of the lipase conformation as these residues were high in preference.

THE PREFERENCE OF CHEMICAL PROPERTIES OF THE PEPTIDES

The functional amino acid properties of the inhibitory peptides can be categorized into hydrophobic-aliphatic, hydrophobic-aromatic, polar-neutral, hydrophilic-acidic, and hydrophilic-basic according to their amino acid constituents (**Figure 3**). In general, the present evidence has demonstrated that pancreatic lipase inhibitor peptides tend to be hydrophobic-aliphatic in nature. Considering most of the bound hotspots of pancreatic lipase inhibitory peptides were prone to be LEU, GLY, ALA, PRO, PHE, and TYR–these hotspots belong to hydrophobic properties except that HIS and SER were hydrophilic and polar-neutral. This suggested that the hydrophobicity nature of these inhibitory peptides tend to promote the interaction with the pancreatic lipase as this enzyme is a soluble globular protein, which must undergo structural changes before it can hydrolyze fat droplets coated with bile salts.

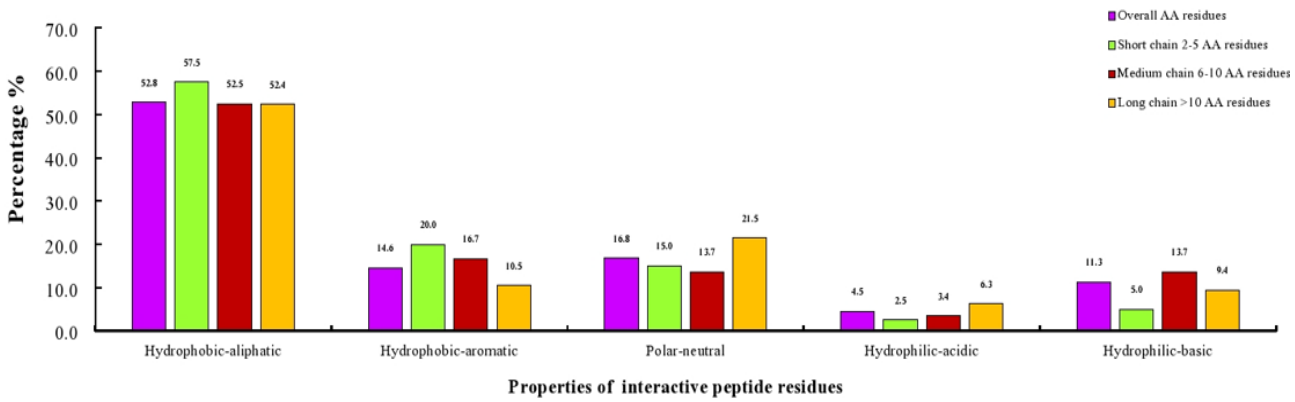

Figure 3. Classification of interactive peptide residue properties of pancreatic lipase inhibitory peptides (Source: Authors' own elaboration)

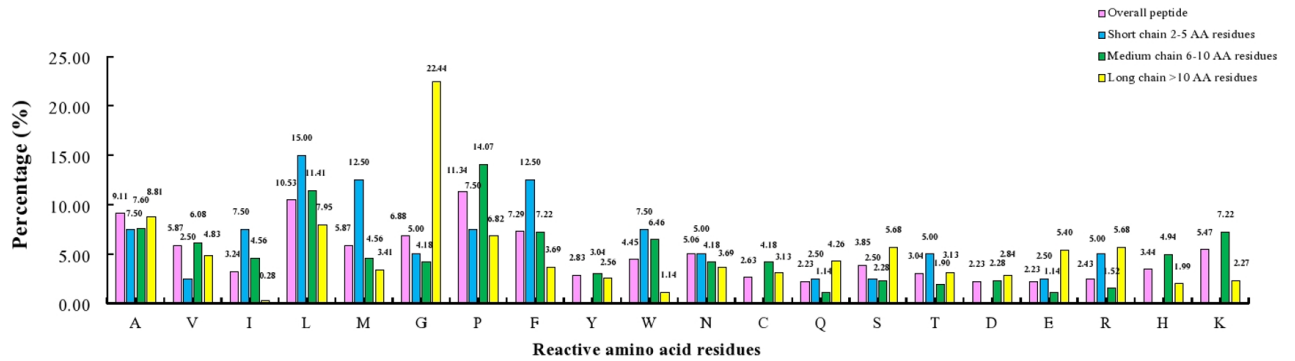

Figure 4. Classification of reactive amino acid residues that presents in interactive peptide residues of pancreatic lipase inhibitory peptides (Source: Authors' own elaboration)

Correspondingly, the binding of colipase and movement of the lipase lid enables open access to the active site triggering the maximal catalytic efficiency of lipase in the presence of bile salt [39]. Moreover, the polar-neutral preference is determined by the presence of SER, which could be accounted for by the lipase active sites, which possess a nucleophilic polar serine, an acid aspartic, and a positively charged histidine. This so-called 'lid' was observed to form an α-helical structure covering the active site. This catalytic process starts with acylation leading to the formation of the acyl-enzyme complex via a nucleophilic attack at the activated serine on the carbonyl C-atom of the substrate ester bond and further diacylation reconstruct the enzyme during the second step of the reaction and releasing the substrate from the serine and thereby finalizing the hydrolysis reaction [40].

THE PREFERENCE OF THE REACTIVE AMINO ACID TYPE IN THE PEPTIDES

As shown in **Figure 4**, structural activity relationship analysis from the overall reactive peptide residues had demonstrated that PRO and LEU were the most favorable amino acid components in the inhibitory peptides, other amino acid substances that may be involved in these peptides were ALA, PHE, and GLY. However, the short chain peptide analysis ascertained that LEU was the most favorable one followed by MET and PHE. Whereas in medium chain peptides, PRO and LEU were commonly present. Meanwhile, the long chain peptide showed that GLY were the most prominent one, followed by ALA, LEU, and PRO. These amino acids were reported to be the top donors and acceptors via C_{α} or $C_{ali}\cdots\pi$ interaction [41]. Therefore, docking analysis was conducted in order to verify the roles of these peptides. The results showed that LEU, MET and PRO tend to bind to the aforementioned hotspots. The details of the interaction are presented in **Supplementary Materials 2**, which demonstrated that hydrophobic interactions and hydrogen bonds were actively involved. However, the significance of PRO and LEU were uncertain when examining the medium peptides as these residues were not involved in any interactions in some peptides. Similar occurrence was found on examination of the long peptides. Therefore, it was suggested that although the type of amino acid residue is important, their positioning along the peptides and the neighboring amino acids could exert important influences especially when a greater number of amino acids are present in the peptide.

PREFERENCE OF THE N- OR C-TERMINAL AMINO ACID RESIDUES IN THE PEPTIDES

Another critical feature of the bioactive peptides was the position of the N- and C-terminal [42]. N- and C-terminals have been reported to be the most flexible regions in the protein structure, playing crucial roles in protein folding, native state stability, final turnover and are essential for enzyme assembly, allosteric, and catalytic properties.

Moreover, modification of a flexible region, such as N- and C-terminals, may affect the stability and flexibility of the protein. These modifications will result in altered biological functions and biochemical properties [43, 44].

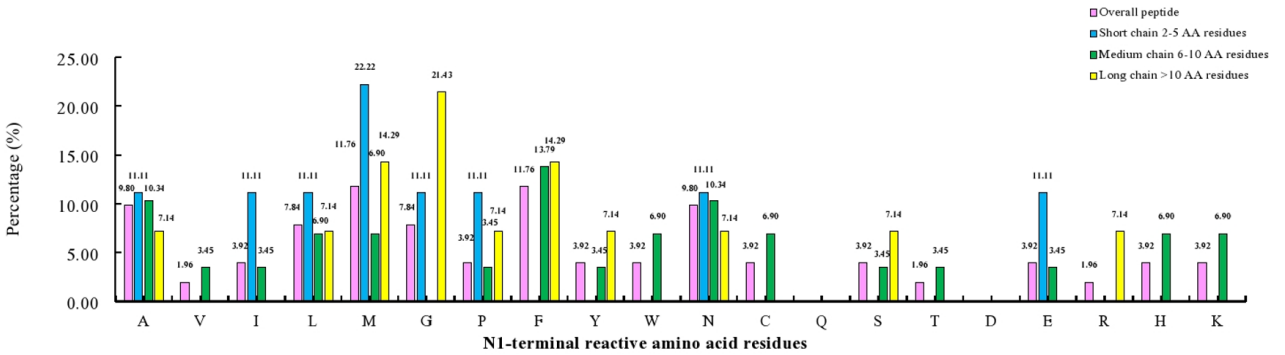

Figure 5. Classification of N1-terminal reactive amino acid residues of pancreatic lipase inhibitory peptides (Source: Authors' own elaboration)

N-Terminal

As shown in **Figure 5**, the N-terminal overall reactive amino acid residues MET, and PHE were the most preferred individual amino acid. In the short chain peptide, MET was the only reactive amino acid residue active in the peptides. Data were acquired via molecular docking studies for short chain peptides containing MET **(**M**M**ML and **M**SNYF) at n position. The results showed that MET is likely to interact with active residues of PHE78 and PHE216 of the enzyme, as well as other residues (TYR115, PRO181, THR256, ARG257, VAL260, and ALA261) (**Supplementary Materials 1**, **Table S1a-b**, and **Figure S1a-b**).

Furthermore, PHE followed by ALA and ASN seemed to be essential amino acids in the N-terminal of medium chain peptides (**Figure 5**). The docking results also demonstrated that PHE (**F**DPFPK and **F**YLGYCDY) had the highest number of interactions in which the active residues of the enzyme (i.e., PHE78, SER153, PHE216, and HIS264) as well as other residues, such as ILE79, TYR115, LEU214, and VAL260, were bound via hydrophobic bonds (**Supplementary Materials 1**, **Table S2a-b**, and **Figure S2a-b**). ASN (**N**PVWKR and **N**PVWKRK) however, exhibited insignificant binding (**Supplementary Materials 1**, **Table S2c-d**, and **Figure S2c-d**); whereas **A**GDDAPR could only bind to PHE78 and TYR115 (**Supplementary Materials 1**, **Table S2e**, and **Figure S2e**), and **A**PFPLR did not show any binding.

The prominent amino acids in the N-terminal of long chain peptides appeared to be GLY, PHE, and MET. Docking analysis was therefore performed on the long chain peptides containing GLY (**G**NPVGGVGHGTTGT and **G**QLGEHGGAGMG), PHE (**F**FRSKLLSRGAAAAKGALLPQYW and **F**VVAEQAGNEEGFE) and MET (**M**LPLMLPFTMGY and **M**SKFLPLPLMFY). However, only two peptides were found to interact with the active sites; **G**QLGEHGGAGMG was bound to PHE78 and TYR115 (**Supplementary Materials 1**, **Table S3b**, and **Figure S3b**) whereas **M**SKFLPLPLMFY was bound at PHE78, ILE79 and PHE216 (**Supplementary Materials 1**, **Table S3f**, and **Figure S3f**).

The data from the present investigation suggested that in the position of n, MET (hydrophobic–aliphatic) in short peptide whereas PHE (hydrophobic–aromatic) in medium peptides play a major role in pancreatic lipase inhibitory actions. MET-aromatic residue bridges were reported to play crucial roles in protein-protein interactions. For examples, it mediates electron transfer between proteins, it stabilizes and protects the protein structures. It also provides motifs for molecular recognition [45].

According to the reported peptide-based models, MET could be a good redox mediator in the electron transfer between aromatic sites [46]. MET was also reported to provide additional stabilization of 1-1.5 kcal/mol in its hydrophobic interaction with protein motifs [47]. The localization of sulphur residues in the MET with the aromatic-containing residues (TRP, TYR, and PHE) in close proximity (approximately 5.5 Å between the sulphur with the ring center) was widely reported in different protein structures based on a number of atomistic molecular dynamic simulations [48]. This is supported by the data in which MET tends to bind to the two important hotspots of PHE78 and PHE216. It was also reported that the interaction of Aro-Met-Aro is occurs relatively frequent and most of these bridges contain at least one PHE [45].

Apart from MET, PHE is also important for protein-protein interactions. It was suggested that peptide design using this residue could modulate protein functions effectively [49]. A systematic study of phenylalanine in the CH···π, OH···π, NH···π, and cation···π interactions has been performed [50]. It was also reported that PHE was one of the well-known CH···π interaction acceptors in which its aromatic side chain usually interacted with other aromatic side-chains via stacking interactions [41]. In addition, this hydrophobic PHE can be involved in the binding of hydrophobic ligands [51]. Thus, a high number of interactions with the crucial active residues of the enzyme in particularly, PHE78, PHE216, and HIS264, have been observed.

Moreover, the present findings were also supported by [30], where the N-terminal end of these inhibitory peptides with high pancreatic lipase interaction potential could be subjugated by hydrophobic amino acid residues. These interactions are potentially acting on water-lipid interfaces and ultimately promote structural re-orientation of the globular domain on the central β-sheet core of the pancreatic lipase enzyme.

C-Terminal

Figure 6 shows that C-terminal reactive amino acid residues that were present in the overall peptide were LEU, LYS, and ARG. Surprisingly, PRO was present very frequently in the short chain peptides, apart from LEU. The findings from the molecular docking studies demonstrated that the short chain inhibitory peptide, MMM**L**, which has a LEU residue in the C position, was likely to interact with TYR115, SER153, LEU154, ALA179, PRO181, PHE216, and HIS 264 residues of the pancreatic lipase enzyme (**Supplementary Materials 1**, **Table S4a-b**, and **Figure S4a-b**), whereas FDM**L** was only bound to ILE79. As for the RLL**P** short chain inhibitory peptide, which has PRO as targeted amino acid residue in C position, it tended to

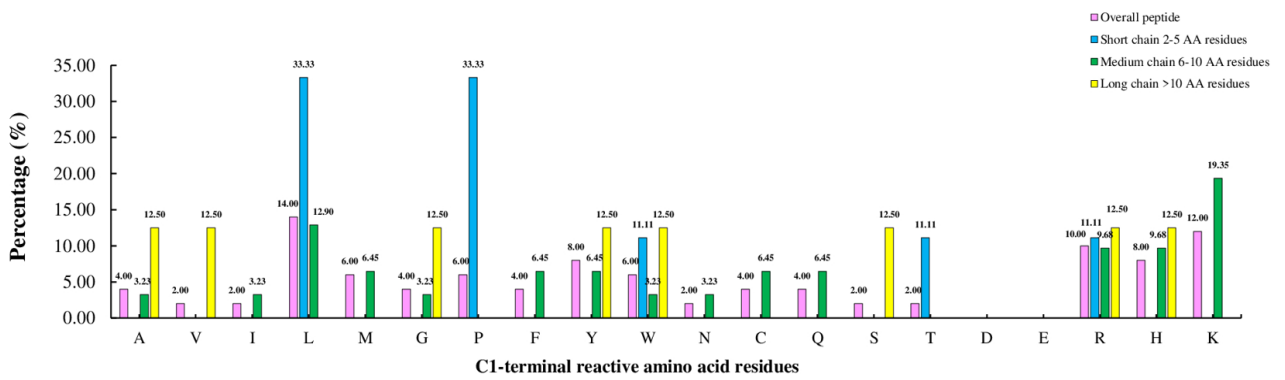

Figure 6. Classification of C1-terminal reactive amino acid residues of pancreatic lipase inhibitory peptides (Source: Authors' own elaboration)

interact with the TRP253, THR256 and VAL260 residues (**Supplementary Materials 1**, **Table S4c**, and **Figure S4c**).

In addition, LYS was the most frequently reactive amino acid residues found in medium chain peptide followed by LEU. Of great hypothetical interest, it was found that in the medium chain peptides i.e., LAPSTI**K** and KVEGDL**K**, which have a binding affinity of -8.0 and -7.9 Kcal/mol, the LYS residue in C position of the LAPSTI**K** inhibitory peptides interacted predominantly with TYR115, ALA179, PRO181 and PHE216 via an hydrophobic interaction and further interacted with the TRY115 residue of the pancreatic lipase enzyme via hydrogen bonding at a distance of 2.81 Å (**Supplementary Materials 1**, **Table S5a**, and **Figure S5a**). Likewise, KVEGDL**K** was essentially hydrophobically and interacted with the PHE78, TYR115, PRO181, ILE210, PHE216, and HIS264 residues (**Supplementary Materials 1**, **Table S5b**, and **Figure S5b**). As for the contribution of LEU, it was observed that only six out of 10 peptides had an interaction with lipase which involved the C-terminal LEU (**Supplementary Materials 1**, **Table S5c**, and **Figure S5l**). GMAGGPPL**L** had the most interactions with the lipase (i.e. Phe78, Ile79, Arg257, Val260, His264, and Leu265), of which some are the aforenamed hotspots.

However, the ALA, VAL, GLY, TYR, TRP, ARG, and HIS residues were equally present in the C-terminal from the long chain peptides. 12 long chain inhibitory peptides were also selected to further understand the interactive nature with the pancreatic lipase enzyme (**Supplementary Materials 1**, **Table S6a-l**, and **Figure S6a-l**). These peptides showed no significant or negligible binding to the enzyme. For example, the ALA residue at the C position of the NALKCCHSCP**A** residue tended to bind with only THR116 via a hydrogen bond at a distance of 2.86 Å while THR116 and GLY114 residues were observed to have a hydrophobic interaction with a binding affinity at -10.4 Kcal/mol (**Supplementary Materials 1**, **Table S6a**, and **Figure S6a**). No interactions were identified among the VAL (GEHGGAGMGGGQFQP**V**), GLY (GQLGEHGGAGM**G** and LAAVEALSTN**G**) inhibitory peptides with the pancreatic lipase enzyme (**Supplementary Materials 1**, **Table S6b** to **Table S6d**, and **Figure S6b** to **Figure S6d**).

From the data obtained, LEU at C-terminal in short and medium peptides possibly play a major role in inhibiting pancreatic lipase followed by LYS in medium peptides. However long peptides did not show any specific preference in the type of amino acid. From the CH···π interaction in the proteins studied in [41], it was concluded that LEU was the most efficient donor residue in the C_{ali}-H···Aro-π interactions. When the researchers in [52] studied the structural basis of peptide-protein binding, LEU exhibited a very high frequency compared to other peptide residues, hence, they concluded that peptide interface hotspots could be enriched using this residue. Therefore, their report would support the findings of the current study in which LEU tended to bind to PHE78, TYR115, PRO181, PHE216, and HIS 264.

Similarly, the LEU and LYS amino acid were also reported to be the preferential donor in C_{α}-H···Aro-π interactions. On the other hand, it was also reported that the LYS amino acid played an essential part in protein-protein associations [53]. Our data also supported the view that this residue binds to aromatic side chains of PHE78, TYR115, PRO181, PHE216, and HIS264. Apart from pi stacking, the ε-amino group of lysine residues could also contribute to hydrogen bonding, which is also important in the catalysis of ligand contact that may be critical for substrate specificity [54]. This view is further supported by the finding that the LYS of the LAPSTI**K** peptide interacted with TYR115 via a hydrogen bond (**Table 5Sa**).

CONCLUSION AND PERSPECTIVES

The utilization of peptides for therapeutic purposes has evolved recently and continues to evolve in obesity preventive paradigms development. In the past century, the food-based supplement industry has been dominated using small molecules which are distinguished by high attrition rates in order to fulfil several requirements i.e., pharmacokinetics, pharmacodynamics, toxicity and safety issues which has resulted in more than 90% of the initiative efforts having failed to achieve the marketplace. Peptides are a unique class of biochemical compounds, which are molecularly poised between small molecules and proteins but are biochemically and therapeutically distinct from each other. They act as intrinsic signaling molecules in many physiological functions and are potentially useful for therapeutic intervention as they closely mimic natural pathways. These peptides are readily degraded within the human body and therefore their utilization is relatively safe [55]. From the safety point of view, inhibitory peptides derived from natural substrates are much applicable than chemically synthesized compounds. Moreover, natural-derived lipase inhibitors are relatively cheap and widely available although inhibitory effectiveness and active compounds are difficult to determine. Hence, screening novel lipase inhibitors from plant origin with minimal side effects has been a hotly debated topic in research activities [3].

Data from this investigation has demonstrated that the majority of these inhibitory peptides were hydrophobic nature. These results were consistent with the investigation reported in [39], which studied the hydrophobicity of the tilted peptides and provided evidence for the hydrophobic nature of the inhibitory peptides. Amino acid residues such as PHE78, HIS152, and PHE216 could be classified as the inhibitory residues while the SER153, ASP80, and HIS264 could participate as catalytic sites for the pancreatic lipase enzyme, that are responsible for the disruption of lipid interfaces [11, 56, 57]. In addition, these aforementioned authors had also demonstrated that when the long peptide was hydrophobic enough to penetrate among the acyl chains, it was likely to perturb their parallel stacking and truncated the accessibility of enzymes to hydrophobic substrates. However, in this investigation, the presence of other unreported amino acid residues such as ALA, ARG, GLU, GLY, ILE, LEU, LYS, PRO, TRP, TRY, and VAL in both N- and C-terminals suggested that some structural features are required. They may act with essential acylable residues in the catalytic mechanism of the enzyme to allow generation of an accessible surface area in the open and closed lipase lid domain that can interact with the protein core in the crystal structure of the pancreatic lipase enzyme [34]. For instance, the PRO-LEU-PHE is responsible for the hydrophobic groove and ILE-LYS-GLU is responsible for forming the β5-loop of the pancreatic lipase enzyme [39].

The functional importance of the C-terminal domain in pancreatic lipase was to interact with colipase but also to form a reciprocal interaction with the N-terminal domain as most of the helices in protein three dimensional structures are amphipathic and lie flat between a hydrophobic-hydrophilic interface. This facilitates such fragments to adsorb onto or to penetrate the hydrophobic interfaces, which could ultimately destabilize the packing of acyl chains. Such an activation mechanism seems to underlie lipase catalysis. Therefore, the open lid is a crucial component of the active site for interaction with procolipase. Together, these components they form the lipid-water interface binding site. Thus, reorganization of the lid structure could provoke a second drastic conformational change in an active site loop, which in turn creates the oxyanion hole [58]. In the case of the physiological situation of pancreatic lipase, the amphipathic substances such as LYS, TYR, and TRP; and other proteins or phospholipids, prevent the binding of pancreatic lipase to the lipid-water interface. Hence, pancreatic lipase needs a small protein partner, namely colipase also secreted by the exocrine pancreas, which allows the enzyme to bind to the bile salt-covered interface. It was also predicted that the lid movement was closely related to the paradox of interfacial activation. This would be linked to the increase of lipase activity in the presence of a lipid-water interface as this hydrophobic surface helps to bring the catalytic domain of lipase into close contact with the interface [59]. Therefore, there is now a clear consensus that lipase activation is in part dependent on the displacement of the lid that opens the active site, and this process goes with a modification of the lid secondary structure. Indeed, this statement further supports the hypothesis that the lid not only plays a significant role in modulating the enzyme activity but also on its enantioselectivity, specificity, and stability of lipase enzymes [39, 60].

To this end, this paper aims to accentuate the pivotal role of hotspots within pancreatic lipase enzymes that tend to be bound by the identified inhibitory peptides. Correspondingly, the data demonstrated that hydrophobic-aliphatic and polar-neutral interactions were the fundamental property for the inhibitory peptides, where hydrophobic bonding to the pancreatic lipase enzyme was essential. It was also recognized that different peptide lengths seem to interact with their own preference for various amino acids beyond the bounds of the amino acids at the N- and C-terminals. Moreover, this paper also intended to highlight the general features of the pancreatic lipase inhibitory peptides to provide some direction for future peptide-based drugs design and discovery. These data allowed us to develop strategies in designing inhibitory peptides by identifying the recognition processes for their binding mechanisms as well as how the stabilization between peptide and lipase is established. It should be noted that an in-depth structural characterization of peptide-lipase complexes is also required. Nevertheless, in the present investigation, our team was managed to procure only 176 selected identifiable peptides from currently available databases. Although, this data pool might not be adequate to illustrate the substantive activities from all the pancreatic lipase inhibitory peptides and represents a major limitation in this study. Despite that, we believed that the current reporting data could provide guidance and a foundation for practical knowledge for future peptide based anti-obesity drug designs.

Author contributions: YCT: data analysis; **YCT & C-YG:** writing-original draft preparation, conceptualization, & data validation; **YCT, C-YG, & MHS:** methodology; **YCT, C-YG, & EJJ:** writing-review and editing; **YCT, PGY, AA, & MHA:** data collection & analysis; & **PGY, AMR, AA, & VM:** software. All authors have agreed with the results and conclusions.

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APPENDIX A

Table A1. List of pancreatic lipase inhibitor peptides with their production methods, sources, & IC50/activity values

Peptide sequence	Production method	Source	IC_{50}/activity	Reference
EQGFLPGPEESGR	Synthetic	Millet grains	76.81±18.33 µg/mL	[7]
YGNPVGGVGH			102.25±1.40 µg/mL	
GNPVGGVGHGTTGT			104.21±4.23 µg/mL	
GEHGGAGMGGGQFQPV			62.32±4.44 µg/mL	
GQLGEHGGAGMG			60.62±17.20 µg/mL	
RLARAGLAQ			97.31±28.57 µg/mL	
IIAPPER	Fermentation	Tropical house cricket (*gryllodes sigillatus*), mealworm- (*tenebrio molitor*), & desert locust (*schistocerca gregaria*)	143.17 µM	[22]
LAPSTIK			144.05 µM	
VAPEEHPV			114.25 µM	
KVEGDLK			146.60 µM	
NYVADGLG			143.17 µM	
AAAPVAVAK			64.35 µM	
YDDGSYKPH			5.38 µM	
AGDDAPR			110.61 µM	
GKDAVIV			110.59 µM	
AIGVGAIER			56.47 µM	
FDPFPK			129.11 µM	
YETGNGIK			107.80 µM	
ELPPHFL	Enzymatic-alcalase, trypsin	Sea cucumber (*C. frondose*)	75.52%	[17]
APFPLR			60.75%	
LNLDLLR			93.08%	
LNFEPR			72.32%	
TTDVLR			94.14%	
MANLQR			82.58%	
MMML, FDML, HLPGRG, AAGF, MSNYF, FLWPEYGAL	Enzymatic-alcalase, pronase-E	Cow casein hydrolysates	12.5±0.404 mg/mL	[23]
		Camel casein hydrolysates	12.7±0.346 mg/mL	
PAGNFLP, MLPLMLPFTMGY, LRFPL	Enzymatic-pepsin, chymotrypsin, & trypsin	Camel casein hydrolysates	CWPH 19-0.46 mg/mL	[25]
FYLGYCDY	Molecular docking	De-oiled rice bran	2.8360±0.2022 µg/ml	[8]
SPFWNINAH	CABS-dock computational model	Oat bran	85.4±3 µM	[9]
NVQR, AQMACPHL, VAPAGHAVT, PHHCDAEAI, HSDDDGQIR, TATAVV, LQR, GTIT	Molecular docking of proteins & peptides from theobroma cocoa L	Cocoa protein (CP) hydrolysates	1.38 mg/mL	[10]
FFRSKLLSRGAAAAKGALLPQYW, RCMAFLLSDGAAAAQQLLPQYW, RPAQPNYPWTAVLVFRH	Chemically synthesized	Cumin seed	54.6%, 50.1%, & 22.6%	[11]
DFGIASF, LAAVEALSTNG	Consecutive chromatography & mass spectrometry	Brewer's spent grain	0.04 mg/mL	[12]
RLLP	Synthetic peptide- LC-MS/MS	Hazelnut (*corylus heterophylla fisch*) protein hydrolysates	Pancreatic lipase inhibition rate of RLLPH was above 40% at concentration of 80 mM	[13]
DIVDKIEI	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) & quadrupole time-of-flight mass spectrometry (Q-TOF MS/MS)	Tuna peptide	Significantly decreased triglyceride level by 2 folds	[18]
EITPEKNPQLR, RKQEEDEDEEQQRE	Soy peptides were isolated from hydrolysates of purified β-conglycinin by co-immunoprecipitation & identified using LC-MS/MS	Soybean β-conglycinin	Inhibited fatty acid synthase 27.4 µm, 16.5 µm	[14]
INEGSLLLPH, FVVAEQAGNEEGFE, SGGGGGGVAGAATASR, GSGGGGGGGFGGPRR, INEGSLLLPH, GGYQGGGYGGNSGGGYGNRG, GGSGGGGGSSSGRRP, GDTVTVEFDTFLSR	Enzymatic–in-vitro hydrolyzed under gastrointestinal conditions	Fermented bean (*phaseolus vulgarisL.*) seeds	Highest lipase inhibitory activity is 1.19 mg/ml	[15]
HTVMILFK, APYVMILF	Enzymatic-Purafect® with a final degree of hydrolysis of about 14.5%	Shark-smooth hound viscera protein	Decreased lipase activity from 180 U/mL in high fat-fed rats to about 155 U/mL	[19]

Table A1 (Continued). List of pancreatic lipase inhibitor peptides with their production methods, sources, & IC50/activity values

Peptide sequence	Production method	Source	IC_{50}/activity	Reference
Alcalase: AEWLHDWKL, ALWGAGGGGLGLSSGR, AVVSPLKPCC, CFLPLPLLK, DNLMPQFM, FCLPLPLLK, FMFFGPQ, GMAGGPPLL, HCPVPDPVRGL, KDLWDDFKGL, KFQWGY, LLPAPPLL, LTMPQWW, MMHDFLTLCM, MSKFLPLPLMFY, SQDWSFY, WGLWDDMQGL, WNWGWLLWQL, YWYPPK, YWYPPQ Bromelain: TLMPQWW, MPSKPPLL, AVVSPLKPCC	Enzymatic-alcalase, bromelain	Camel milk proteins	Alcalase- 0.029±0.0008 mg/ml Bromelain- 0.029±0.0003 mg/ml	[24]
LKCCHSCPA, LNNPSVCDCDCMMKAAR, NPVWKRK, CANPHELPN	Enzymatic-trypsin, alcalase, pepsin, papain, & protamex	Spirulina platensis	Inhibitory effects on pancreatic lipase; Trypsin- 27.24±3.71%, Alcalase- 51.29±0.06, Pepsin- 50.61±1.94, Papain- 27.24±3.71 & Protamex- 66.23±1.71	[21]
NALKCCHSCPA	Enzymatic-trypsin, alcalase, pepsin, papain, & protamex	Dried seaweed sample of spirulina platensis	Papain>Simvastatin (32.67%) Pepsin>Simvastatin (72.00%) Alcalase>Simvastati (32.67%) Trypsin>Simvastatir (45.38%) Protamex>Simvastati (32.67%)	[21]
EF	Nuclear magnetic resonance and electrospray ionization–mass (ESI–MS) spectroscopy	Onion (*allium cepa L. cv. sunpower*)	IC_{50} not indicated; increased hepatic lipogenesis, accounting for about 25% of fatty acid, is a key feature associated with non-alcoholic fatty liver disease (NAFLD)	[16]
VIAPW IRWWW	Enzymatic-protein hydrolysate of miiuy croaker (*m. miiuy*) muscle	Miiuy croaker (*miichthys miiuy*) muscle	IC_{50} not indicated: VIAPW & IRWWW could play their hypolipidemic activities in HepG2 cells through regulation of AMPK pathway & act as hypolipidemic nutrient ingredients	[13]
LNNPSVCDCDCMMKAAR, NPVWKR, CANPHELPN, LKCCHSCPA	Enzymatic- trypsin, alcalase, pepsin, papain and protamex	Spirulina platensis	IC_{50} not indicated: All 4 peptides exhibited inhibitory effects on 3T3-L1 pre-adipocytes proliferation (32.29–60.08%). Moreover, NPVWKRK & CANPHELPNK also significantly decreased accumulation of triglyceride at 600 µg/mL ($p<0.05$), up to 23.7% & 19.5%.	[21]

APPENDIX B

Table B1. Summary of active peptide fragments & interactive peptide & pancreatic lipase bound residues on Pepsite2 analysis

Peptide	p-value	Peptide interactive residues	Lipase (1ETH) bound residues
Short (2-5 amino acid residues)			
MMML	0.006827	met-1, met-2, met-3, leu-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, ALA261, HIS264
FDML	0.096660	phe-1, met-3, leu-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
AAGF	0.010860	ala-1, ala-2, gly-3, phe-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, HIS264
MSNYF	0.026910	met-1, ser-2, asn-3, phe-5	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
LRFPL	0.035500	phe-3, pro-4, leu-5	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, HIS264
NVQR	0.028240	asn-1, val-2, gln-3, arg-4	ASP80, LYS81, GLU84, TRP253, ARG257
LQR	0.320500	leu-1, gln-2	PHE78, TYR115, SER153, PHE153, ALA261, HIS264
GTIT	0.030550	gly-1, thr-2, ile-3, thr-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
RLLP	0.031060	leu-2, leu-3, pro-4	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, HIS264
EF	0.196500	glu-1, phe-2	PHE78, TYR115, SER153, PHE216
VIAPW	0.003606	ile-2, ala-3, pro-4, trp-5	GLY77, PHE78, ILE79, TYR115, SER153, LEU154, PRO181, PHE216, HIS264
IRWWW	0.158000	ile-1, arg-2, trp-3, trp-4	GLY77, PHE78, ILE79, TYR115, SER153, LEU154, PHE216, HIS264
Medium (6-10 amino acid residues)			
RLARAGLAQ	0.240900	arg-1, leu-7, ala-8, gln-9	PHE78, LYS81, GLU84, TYR115, SER153, PHE216, TRP253, ALA261, HIS264
IIAPPER	0.038890	ile-1, ile-2, ala-3, pro-4	PHE78, TYR115, SER153, PRO181, PHE216, HIS264
LAPSTIK	0.037150	pro-3, ser-4, thr-5, ile-6, lys-7	PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
VAPEEHPV	0.172900	ala-2, pro-3, glu-4, his-6	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, HIS264
KVEGDLK	0.608000	gly-4, leu-6, lys-7	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
NYVADGLG	0.253200	asn-1, tyr-2, val-3, leu-7, gly-8	GLY77, PHE78, ASP80, LYS81, GLU84, TYR115, SER153, LEU154, PHE216, TRP253, ARG257, HIS264
AAAPVAVAK	0.087410	val-5, ala-6, val-7, ala-8, lys-9	GLY77, PHE78, TYR115, SER153, LEU154, PRO181, PHE216, HIS264
YDDGSYKPH	0.294300	tyr-1, lys-7, pro-8, his-9	PHE78, LYS81, TYR115, SER153, PHE216, TRP253
AGDDAPR	0.339500	asp-4, ala-5, pro-6	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, HIS264
GKDAVIV	0.202700	asp-3, ala-4, val-5, ile-6	GLY77, PHE78, TYR 115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, HIS264
AIGVGAIER	0.329400	ala-1, ile-2, gly-3, arg-9	GLY77, PHE78, TYR115, SER153, LEU154, PRO181, PHE216, TRP253, HIS264
FDPFPK	0.003058	asp-2, pro-3, phe-4, pro-5, lys-6	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
YETGNGIK	0.282100	gly-4, asn-5, ile-7, lys-8	GLY77, PHE78, TYR115, SER153, LEU154, PRO181, PHE216, HIS264
ELPPHFL	0.009946	glu-1, leu-2, pro-3, pro-4, his-5	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
APFPLR	0.572400	phe-3, pro-4, leu-5, arg-6	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
LNLDLLR	0.282500	leu-1, asn-2, leu-3, asp-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, HIS264
LNFEPR	0.235700	leu-1, asn-2, phe-3, glu-4	PHE78, TYR115, SER153, ALA179, GLU180, PRO181, PHE216, HIS264
TTDVLR	0.337500	thr-1, thr-2, asp-3, val-4	GLY77, PHE78, ILE79, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
MANLQR	0.050140	met-1, ala-2, asn-3, leu-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, HIS264
HLPGRG	0.056320	his-1, leu-2, pro-3, gly-4	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
FLWPEYGAL	0.236900	phe-1, leu-2, trp-3, pro-4	GLY77, PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
PAGNFLP	0.150600	ala-2, gly-3, asn-4, phe-5	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
FYLGYCDY	0.158700	phe-1, tyr-2, leu-3, gly-4, tyr-5	GLY77, PHE78, TYR115, SER153, LEU154, PRO181, PHE216, HIS264
SPFWNINAH	0.521200	ser-1, pro-2, phe-3, trp-4, his-9	GLY77, PHE78, ILE79, LYS81, GLU84, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, TRP253, HIS264
AQMACPHL	0.030180	met-3, ala-4, cys-5, pro-6, his-7	PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
VAPAGHAVT	0.236900	val-1, ala-2, pro-3, ala-4	PHE78, TYR115, HIS152, SER153, LEU154, ALA179, PRO181, PHE216, ALA261, HIS264
PHHCDAEAI	0.275200	pro-1, his-2, his-3, ile-9	PHE78, LYS81, GLU84, TYR115, SER153, LEU154, ALA179, PHE216, TRP253, HIS264
HSDDDGQIR	0.185400	his-1, ser-2, gln-7, ile-8, arg-9	GLY77, PHE78, LYS81, GLU84, SER153, LEU154, PHE216, TRP253, HIS264
TATAVV	0.048440	ala-2, thr-3, ala-4, val-5	GLY77, PHE78, TYR115, SER153, LEU154, PRO181, PHE216, HIS264
DFGIASF	0.022110	phe-2, ile-4, ala-5, ser-6, phe-7	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
DIVDKIEI	0.368000	asp-1, ile-2, val-3, lys-5	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, HIS264
INEGSLLLPH	0.069920	ans-2, leu-7, leu-8, pro-9, his-10	GLY77, PHE78, LYS81, GLU84, TYR115, SER153, LEU154, PHE216, TRP253, HIS264

Table B1 (Continued). Summary of active peptide fragments & interactive peptide & pancreatic lipase bound residues on Pepsite2 analysis

Peptide	p-value	Peptide interactive residues	Lipase (1ETH) bound residues
HTVMILFK	0.011460	val-3, met-4, ile-5, leu-6, phe-7, lys-8	GLY77, PHE78, ILE79, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
APYVMILF	0.058750	val-4, met-5, ile-6, leu-7, phe-8	GLY77, PHE78, ILE79, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
AEWLHDWKL	0.243000	trp-3, leu-4, his-5, trp-7, lys-8	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
AVVSPLKPCC	0.110500	ala-1, val-2, val-3, cys-9, cys-10	GLY77, PHE78, LYS81, TYR115, SER153, LEU154, ALA179, GLU180, PRO181, PHE216, TRP253, HIS264
CFLPLPLLK	0.059540	cys-1, leu-5, pro-6, leu-7, lys-9	GLY77, PHE78, LYS81, TYR115, SER153, LEU154, PHE216, TRP253, HIS264
DNLMPQFM	0.080400	met-4, pro-5, phe-7, met-8	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
FCLPLPLLK	0.049920	phe-1, cys-2, leu-3, pro-4, lys-9	PHE78, LYS81, GLU84, TYR115, SER153, PRO181, PHE216, TRP253, HIS264
FMFFGPQ	0.017190	phe-1, met-2, phe-3, phe-4, gly-5	GLY77, PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
GMAGGPPLL	0.251600	pro-6, pro-7, leu-8	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, HIS264
KDLWDDFKGL	0.110800	lys-1, phe-7, lys-8, gly-9, leu-10	GLY77, PHE78, LYS81, GLU84, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, TRP253, HIS264
KFQWGY	0.042020	lys-1, phe-2, trp-4, gly-5, tyr-6	GLY77, PHE78, ILE79, TYR115, SER153, LEU154, PRO181, PHE216, HIS264
LLPAPPLL	0.081780	ala-4, pro-5, pro-6, leu-7	GLY77, PHE78, TYR115, SER153, ALA179, PRO181, PHE216, HIS264
LTMPQWW	0.014160	thr-2, met-3, pro-4, gln-5, trp-6	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, ALA261, HIS264
MMHDFLTLCM	0.053480	met-1, met-2, his-3, leu-8, cys-9, met-10	GLY77, PHE78, LYS81, GLU84, TYR115, SER153, LEU154, PHE216, TRP253, THR256, HIS264
SQDWSFY	0.365600	trp-4, ser-5, phe-6, tyr-7	GLY77, PHE78, ILE79, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
WGLWDDMQGL	0.170700	trp-1, gly-2, leu-3, trp-4, gln-8	GLY77, PHE78, LYS81, TYR115, SER153, LEU154, PRO181, PHE216, TRP253, HIS264
WNWGWLLWQL	0.183700	trp-1, asn-2, trp-3, trp-5, gln-9	GLY77, PHE78, LYS81, TYR115, SER153, LEU154, PHE216, TRP253, HIS264
YWYPPK	0.006533	trp-2, tyr-3, pro-4, pro-5, lys-6	PHE78, TYR115, SER153, PRO181, PHE216, HIS264
YWYPPQ	0.021620	tyr-3, pro-4, pro-5, gln-6	PHE78, TYR115, SER153, PRO181, ILE210, PHE216, ALA261, HIS264
TLMPQWW	0.047010	leu-2, met-3, pro-4, trp-6, trp-7	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, PHE216, ALA261, HIS264
MPSKPPLL	0.038290	ser-3, lys-4, pro-5, pro-6, leu-7	GLY77, PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
AVVSPLKPCC	0.110500	ala-1, val-2, val-3, cys-9, cys-10	GLY77, PHE78, LYS81, TYR115, SER154, LEU154, ALA179, GLU180, PRO181, PHE216, TRP253, HIS264
LKCCHSCPA	0.032990	lys-2, cys-3, cys-4, pro-8, ala-9	PHE78, LYS81, GLU84, TYR115, SER153, PHE216, TRP253
NPVWKRK	0.036640	asn-1, pro-2, val-3, trp-4, lys-5	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, HIS264
CANPHELPN	0.187900	cys-1, leu-7, pro-8, asn-9	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, TRP253, HIS264
NPVWKR	0.013300	asn-1, pro-2, val-3, trp-4, lys-5	PHE78, TYR115, SER153, LEU154, ALA179, PHE216, HIS264
YGNPVGGVGH	0.148200	tyr-1, asn-3, pro-4, val5, his-10	GLY77, PHE78, ILE79, LYS81, GLU84, TYR115, SER153, LEU154, PRO181, PHE216, TRP253, HIS264
Long (>10 amino acid residues)			
EQGFLPG/ PEESGR	0.098530/ 0.446800	phe-4, leu-5, pro-6, gly-7, glu-10, ser-11, gly-12, arg-13	GLY77, PHE78, LYS81, GLU84, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, TRP253, HIS264
GNPVGGV/ GHGTTGT	0.315900/ 0.260000	asn-2, pro-3, val-4, gly-10, thr-11, thr-12, gly-13	GLY77, PHE78, ILE79, LYS81, GLU84, TYR115, SER153, LEU154, PHE216, TRP253, HIS264
GEHGGAGM/ GGGQFQPV	0.504400/ 0.049100	glu-2, his-3, met-8, gly-9, phe-13, gln-14, pro-15, val-16	GLY77, PHE78, LYS81, GLU84, SER153, LEU154, PHE216, TRP253, HIS264
GQLGEH/ GGAGMG	0.165200/ 0.023870	gly-1, gln-2, leu-3, glu-5, his-6, met-11, gly-12	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, ALA261, HIS264
MLPLML/ PFTMGY	0.006172/ 0.120600	met-1, leu-2, pro-3, leu-4, met-5, met-10, gly-11, tyr-12	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, THR256, HIS264,
FFRSKLLSRG/ AAAAKGALLP/ QYW	0.143200/ 0.241900/ 0.300100	phe-1, phe-2, arg-3, ser-4, lys-5, ala-12, ala-13, ala-14, lys-15, gln-21, tyr-22	GLY77, PHE78, LYS81, GLU84, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, TRP253, HIS264,
RCMAFLLSDG/ AAAAQQLLPQ/ YW	0.118900/ 0.169700/ 0.228600	met-3, ala-4, phe-5, leu-6, leu-7, ala-11, ala-12, ala-13, gln-20, tyr-21, trp-22	GLY77, PHE78, TYR115, SER153, LEU154, ALA179, GLU180, PRO181, PHE216, HIS264
RPAQPNYPWT/ AVLVFRH	0.223600/ 0.060420	pro-2, ala-3, gln-4, thr-10, ala-11, val-12, leu-13, val-14, phe-15	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, TRP253, ALA261, HIS264
LAAVEA/ LSTNG	0.157200/ 0.040400	leu-1, ala-2, ala-3, val-4, ser-7, thr-8, asn-9, gly-10	GLY77, PHE78, TYR115, SER153, LEU154, ALA179, GLU180, PRO181, PHE216, HIS264
EITPEK/ NPQLR	0.119600/ 0.078880	ile-2, thr-3, pro-4, lys-6, asn-7, pro-8, gln-9	PHE78, TYR115, SER153, ALA179, GLU180, PRO181, PHE216, ALA261, HIS264
RKQEEDE/ DEEQQRE	0.029740/ 0.046850	arg-1, lys-2, gln-3, glu-4, asp-6, glu-7, asp-8, glu-10, gln-11, gln-12, arg-13	ARG38, TYR50, LYS81, GLU83, GLU84, TRP253

Table B1 (Continued). Summary of active peptide fragments & interactive peptide & pancreatic lipase bound residues on Pepsite2 analysis

Peptide	p-value	Peptide interactive residues	Lipase (1ETH) bound residues
FVVAEQA/ GNEEGFE	0.116100/ 0.751200	phe-1, val-2, val-3, ala-4, gly-8, asn-9, glu-11	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
SGGGGGGV/ AGAATASR	0.482600/ 0.153500	ser-1, gly-2, val-8, ala-9, ala-13, thr-14, ala-15, ser-16	GLY77, PHE78, LYS81, GLU84, TYR115, SER153, LEU154, PRO181, PHE216, TRP253
GSGGGGG/ GGGGPRR	0.787700/ 0.515900	gly-1, gly-8, phe-10, gly-12, pro-13	GLY77, PHE78, LYS81, GLU84, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, TRP253, HIS264
GGYQGGGYGG/ NSGGGYGNRG	0.457900/ 0.278900	gly-2, gln-4, tyr-8, gly-9, asn-11, ser-12, tyr-16, asn-18, arg-19	GLY77, PHE78, LYS81, GLU84, TYR115, SER153, LEU154, PHE216, TRP253, HIS264
GGSGGGGG/ SSSGRRP	0.988000/ 0.548700	gly-1, gly-8, ser-9, ser-10, ser-11, gly-12	GLY77, PHE78, LYS81, GLU84, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, TRP253, HIS264
GDTVTVE/ FDTFLSR	0.575700/ 0.120000	asp-2, val-4, thr-5, phe8, thr-10, phe-11, leu-12	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
ALWGAGGG/ GLGLSSGR	0.333000/ 0.433000	ala-1, leu-2, trp-3, gly-4, gly-9, leu-10, leu-12, ser-13	GLY77, PHE78, ILE79, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PRO181, PHE216, HIS264
HCPVPD/ PVRGL	0.022270/ 0.290300	pro-3, val-4, pro-5, asp-6 pro-7, val-8, arg-9	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, PHE216, HIS264
MSKFLP/ LPLMFY	0.024240/ 0.005059	met-1, ser-2, lys-3, phe-4, leu-5, leu-7, pro-8, leu-9, met-10, phe-11	GLY77, PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264
LNNPSVCDC/ DCMMKAAR	0.218600/ 0.028070	asn-2, asn-3, pro-4, cys-9, met-12, met-13, lys-14, ala-15, ala-16	GLY77, PHE78, TYR115, HIS152, SER153, LEU154, GLY155, ALA179, GLU180, PRO181, PHE216, TRP253, ALA261, HIS264
NALKCC/ HSCPA	0.084820/ 0.007788	asn-1, ala-2, leu-3, lys-4, his-7, ser-8, pro-10, ala-11	PHE78, TYR115, SER153, LEU154, ALA179, PRO181, PHE216, HIS264