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RESEARCH ARTICLE

Pharmacophore-based virtual screening of bioactive peptides as dipeptidyl peptidase 4 inhibitor for type 2 diabetes mellitus drug candidates

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Abstract

Background: Diabetes is a chronic disease characterised by high blood sugar levels. Glucose that accumulates in the blood without being properly absorbed by the body's cells can cause various organ problems. If diabetes is not properly controlled, various complications can occur that endanger the life of the affected person. **Objective:** To find bioactive peptides that have the potential to inhibit di-peptidyl peptidase 4 (DPP-4) enzyme as anti-diabetes drug candidates. **Method:** This research was carried out using pharmacophore-based virtual screening. **Result:** The validation of the pharmacophore-based virtual screening method showed that model III, which had five pharmacophore features consisting of three Pi interactions, one hydrogen bond donor, and three hydrogen acceptors, was the best pharmacophore model with the values of AUC 0.59; EF 1.2; Se 0.69; Sp 0.94; ACC 0.84; Ya 0.06; and GH 0.2. The screening of the 168,400 short-chain peptides using validated pharmacophore model III gave 51 tetrapeptides as the hits compounds with a pharmacophore fit score of more than 50.0%. **Conclusion:** In total, 51 tetrapeptides were enlisted as potential as anti-diabetes mellitus drug candidates.

Introduction

Diabetes is a chronic disease characterised by high blood sugar levels. Glucose that accumulates in the blood without being properly absorbed by the body's cells can cause various organ problems. If diabetes is not properly controlled, various complications can occur that endanger the life of the affected person (DiPiro *et al*., 2005). Data from the International Diabetes Federation showed that the number of diabetes mellitus (DM) patients globally in 2015 reached 415 million and is expected to continue to increase in 2040 to around 642 million (International Diabetes Federation, 2015).

Diabetes mellitus is one of the main causes of morbidity in COVID-19 patients worldwide (Guo *et al*., 2020). Several studies have shown a higher susceptibility to several infectious diseases in people with diabetes. A retrospective study in Wuhan, China, showed that out of 41% individuals treated for COVID-19, 32% had pre-existing health conditions, with diabetes present in 20% (Guan *et al*., 2020). High blood plasma glucose level is one of the factors that lowers the body's immune system against infection. Therefore, patients with co-morbid diabetes are at high risk of developing COVID-19 and have a poorer prognosis (Guan *et al*., 2020). In COVID-19 patients with comorbid diabetes mellitus, it was found that neutrophil levels and erythrocyte sedimentation rate (ESR) were significantly higher than patients without diabetes. Meanwhile, the levels of lymphocytes, red blood cells, and haemoglobin in patients with co-morbid diabetes were significantly lower than in those without diabetes (Guo *et al*., 2020). This implies that patients with diabetes who contract COVID-19 may face a higher likelihood of experiencing an excessive and unregulated inflammatory reaction

and a state of heightened blood coagulability, potentially leading to a more severe prognosis for COVID-19 (Guo *et al*., 2020).

Dipeptidyl peptidase 4 (DPP-4) inhibitors, used in lowering high blood sugar, also prevent the degradation of active incretin hormones like glucagon-like peptide 1 (GLP-1) (Berger *et al*., 2018). This leads to higher levels of active incretin hormones in the blood, thereby enhancing glycemic regulation mainly by stimulating insulin release in response to glucose and suppressing glucagon secretion from pancreatic alpha and beta cells. The pharmacological blocking of DPP-4 has shown effectiveness in treating type 2 diabetes, with various structurally different DPP-4 inhibitors being used in therapy. The most commonly used are sitagliptin, vildagliptin, saxagliptin, linagliptin, and alogliptin. (Berger *et al*., 2018).

Current treatments for type 2 diabetes mellitus (T2DM) have various limitations, including suboptimal control of postprandial hyperglycemia, increased risk of hypoglycemia, weight gain, gastrointestinal side effects, and oedema (American Diabetes Association, 2018). Classes of drugs that are widely used to treat type 2 diabetes include metformin, sulfonylureas (SU), thiazolidinediones (TZD), and alpha-glucosidase inhibitors (AGI). Metformin is the front-line drug for treating type 2 diabetes, while the next line is SU, TZD, and dipeptidyl peptidase-IV (DPP-4) inhibitors. DPP-4 inhibitors generally have a high tolerability and a low risk of hypoglycemia (American Diabetes Association, 2018). Adverse effects of DPP-4 inhibitors were generally absent for major adverse cardiac events (MACE), nonfatal MI, or stroke (Ou *et al*., 2016). Several studies have shown that DPP-4 inhibitors are also not associated with an increased risk of pancreatic cancer (Zhang *et al*., 2017; Pinto *et al*., 2018).

In recent years, many DPP-4 inhibitors have been produced, which are used in treating type 2 diabetes mellitus. However, there is still little research to produce DPP-4 inhibitors from the peptide group. As one of the macronutrient components, peptides have the advantage of being easily accepted by the body and having minimal side effects. So, developing active peptide inhibitors of DPP-4 as antidiabetic drug candidates is necessary. Previous studies have found DPP-4 inhibitors classified based on their interaction with the residue of the DPP-4 catalytic site: (a) covalent inhibitors and (b) noncovalent inhibitors. Sitagliptin, alogliptin, and linagliptin do not form covalent interactions with catalytic residues (Kim *et al*., 2005; Thomas *et al*., 2008), while vildagliptin and saxagliptin covalently inhibit DPP-4 in a slow and reversible two-step process (Liu, Hu, & Liu, 2012). At the same time, Linagliptin can reduce infarct size after myocardial ischemia. Immunohistological findings support the hypothesis that inhibition of DPP-4 through reduced factor-1 alpha-derived stromal cell division may lead to increased recruitment of CXCR-4+ circulating progenitor cells (Hocher *et al*., 2013).

Virtual screening methods, both pharmacophore modelling and molecular docking, have gained attention in quickly identifying drug guide compounds with relatively lower economic investment costs (Niu *et al*., 2013; Fells *et al*., 2014; Joung *et a*l., 2014; Muttaqin *et al*., 2020). Virtual screening is an advanced computational technique used to sift through a database of chemical compounds to pinpoint potential drug candidates. This method is advantageous in lowering research expenses and accelerating the research process compared to traditional pharmacological screening methods (Tang & Marshall, 2011). The current study aimed to find bioactive peptides that have the potential to inhibit the DPP-4 enzyme as an antidiabetic drug candidate using pharmacophore-based virtual screening.

Methods

Hardware

This study was carried out using a computer unit with the specification of Windows 10 Pro 64-bit operating system, AMD Ryzen 9 3900X CPU @ 3.80 GHz 12 (CPUs) processor, 32 GB of RAM DDR4 memory, and 11 GB GDDR5X dedicated VGA.

Active and decoy set compounds

For validating the virtual screening process, 135 active compounds were sourced as positive controls from the website https://www.ebi.ac.uk/chembl/, with IC50 values ranging from 0 to 4000 nM. Additionally, 2,500 decoy compounds, serving as negative controls, were acquired from https://dude.docking.org.

Small peptides database

Exactly 168,400 short-chain peptides were obtained from the Data of small peptides in SMILES and three-dimensional formats for virtual screening campaigns (Prasasty & Istyastono, 2019).

Pharmacophore-based virtual screening

The pharmacophore-based virtual screening was carried out using the LigandScout 4.3 software. Five statins, including alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin, were used to build the pharmacophore models. The validation was carried out

by applying the active set compound and decoy set compound against the overall pharmacophore features of the pharmacophore models, which will then be used as a guiding feature for selecting compounds from a combination of one to four peptides. The parameters measured are the resulting ROC curve showing an Area Under Curve (AUC) value of more than 0.5 and an Enrichment Factor (EF) value of more than 1.0 (Wolber & Langer, 2005), and also other classic enrichment validation parameters such as selectivity (Se), specificity (Sp), accuracy (ACC), Yield of actives (Ya), dan Goodness of Hit-list (GH) values (Triballeau *et al*., 2005; Wolber & Langer, 2005). The validated pharmacophore models were used for the virtual screening of the short-chain peptides.

Results

Five DPP-4 inhibitors from the statin group (alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin) were used to create model pharmacophores, resulting in ten models for virtual screening, as can be seen in Figure 1.

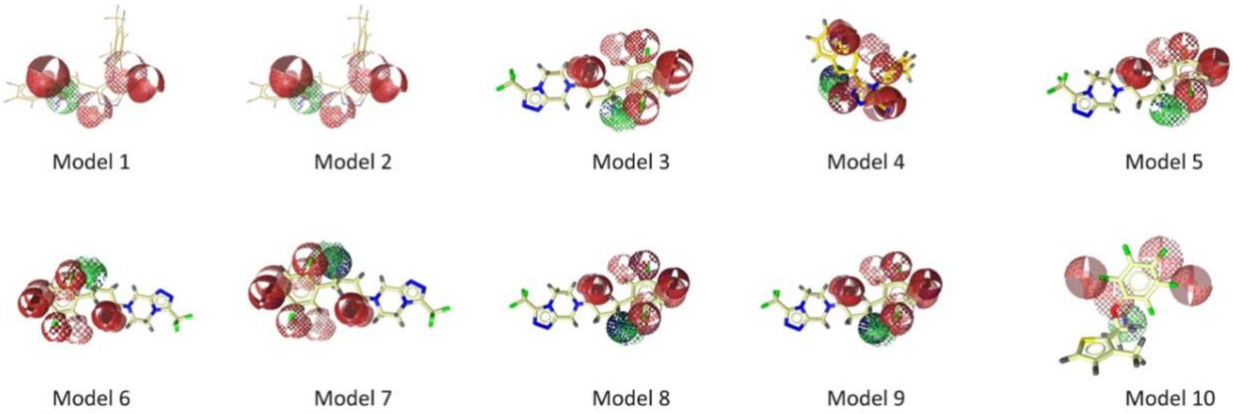

Figure 1: The pharmacophore models obtained from five statin DPP-4 inhibitors

Validation was carried out on the ten pharmacophore models obtained from pharmacophore modelling using the active and the decoy set to obtain receiver operating characteristic (ROC) curves (Table I).

Pharmacophore model III has a total of seven pharmacophore features, consisting of three hydrogen bond acceptors (HBA), one hydrogen bond donor (HBD), and three pi-alkyls (Figure 2).

Table I: Validation of pharmacophore models

Model	Pharmacophore feature(s)			AUC	EF	Se	Sp	Acc	Ya	GH
	HBA	HBD	Pi-Alkyl							
1	3	1	3	0.56	1.0	0.54	0.94	0.87	0.05	0.16
2	3	1	3	0.55	1.0	0.49	0.94	0.92	0.05	0.15
3	3	1	3	0.59	1.2	0.69	0.94	0.84	0.06	0.2
4	3	1	3	10.54	1.0	0.48	0.94	0.94	0.05	0.15
5	4	1	3	0.53	1.0	0.49	0.94	0.89	0.05	0.15
6	4	1	3	0.52	1.0	0.51	0.94	0.84	0.05	0.15
7	4	1	3	0.54	1.0	0.52	0.94	0.85	0.05	0.16
8	4	1	3	0.57	1.1	0.61	0.94	0.83	0.06	0.18
9	4	1	3	0.57	1.1	0.61	0.94	0.83	0.05	0.18
10	4	1	3	0.54	1.0	0.52	0.94	0.85	0.05	0.16

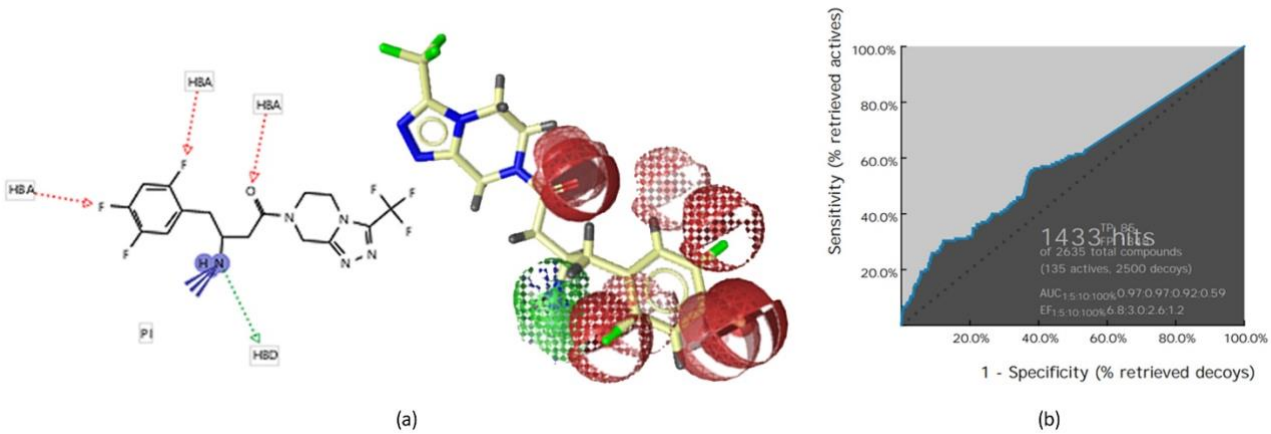

Figure 2: (a) 2D and 3D validated pharmacophore features and (b) ROC curve of pharmacophore model 3

The virtual screening utilised the pharmacophore model III as the validated pharmacophore model, resulting in 51 tetrapeptides as the hits compounds with a pharmacophore fit score of more than 50.0% (Table II).

Table II: Virtual screening results using validated pharmacophore model 3

No	Name	Pharmacophore fit score (%)
1	L-alanyl-L-histidyl-L-isoleucyl-L-arginine	52.48
2	L-alanyl-L-histidyl-L-threonyl-L-arginine	52.39
3	L-alanyl-L-glutamyl-L-prolyl-L-glutamic acid	52.38
4	N-(L-alanyl)-N-methylglycyl-L-prolyl-L-proline	52.38
5	L-seryl-L-arginyl-L-valyl-L-methionine	52.30
6	L-alanyl-L-histidyl-L-prolyl-L-threonine	52.30
7	L-seryl-L-arginyl-L-isoleucyl-L-methionine	52.20
8	L-alanyl-L-aspartyl-L-arginyl-L-leucine	52.19
9	L-alanylglycyl-L-leucyl-L-arginine	52.18
10	L-seryl-L-arginyl-L-histidyl-L-asparagine	52.16
11	L-alanyl-L-alanyl-L-tyrosyl-L-proline	52.15
12	L-seryl-L-arginyl-L-prolyl-L-glutamic acid	52.14
13	L-alanyl-L-cysteinyl-L-tyrosyl-L-arginine	**52.10**
14	L-alanyl-L-aspartyl-L-cysteinyl-L-proline	52.08
15	(S)-3-((S)-2-aminopropanamido)-4-(((S)-1-(((R)-1-carboxy-2-mercaptoethyl)amino)-3-(1H-imidazol-4-yl)-1-oxopropan-2-yl)amino)-4-oxobutanoic acid	51.99
16	(S)-4-(2-((S)-2-aminopropanamido)acetamido)-5-(((S)-1-carboxy-3-(methylthio)propyl)amino)-5-oxopentanoic acid	51.99
17	(S)-4-((S)-2-aminopropanamido)-5-(((2S,3R)-1-(((S)-1-carboxy-3-methylbutyl)amino)-3-hydroxy-1-oxobutan-2-yl)amino)-5-oxopentanoic acid	51.94
18	(S)-4-((S)-2-aminopropanamido)-5-(((S)-3-carboxy-1-(((S)-1-carboxy-2-methylpropyl)amino)-1-oxopropan-2-yl)amino)-5-oxopentanoic acid	51.88
19	L-alanyl-L-aspartyl-L-threonyl-L-proline	51.74
20	L-alanyl-L-histidyl-L-threonyl-L-isoleucine	51.72
21	L-alanyl-L-cysteinyl-L-threonyl-L-lysine	51.72

No	Name	Pharmacophore fit score (%)
22	L-alanyl-L-aspartyl-L-histidyl-L-glutamine	51.66
23	L-alanylglycyl-L-isoleucyl-L-glutamic acid	51.66
24	L-alanyl-L-cysteinyl-L-seryl-L-leucine	51.45
25	L-alanylglycyl-L-leucyl-L-glutamine	51.38
26	L-alanyl-L-histidyl-L-threonylglycine	51.38
27	L-alanyl-L-cysteinyl-L-seryl-L-lysine	51.35
28	L-alanylglycyl-L-leucyl-L-histidine	51.33
29	L-alanylglycyl-L-aspartyl-L-glutamine	51.33
30	L-alanyl-L-histidyl-L-histidyl-L-phenylalanine	51.30
31	L-alanylglycyl-L-lysyl-L-glutamine	51.30
32	L-alanylglycyl-L-tyrosyl-L-serine	51.29
33	L-alanylglycyl-L-leucyl-L-glutamic acid	51.29
34	L-alanyl-L-aspartyl-L-cysteinyl-L-asparagine	51.28
35	L-alanyl-L-isoleucyl-L-cysteinyl-L-serine	51.26
36	L-alanyl-L-phenylalanyl-L-leucyl-L-arginine	51.25
37	L-alanyl-L-histidyl-L-valyl-L-leucine	51.25
38	L-alanyl-L-histidyl-L-threonyl-L-phenylalanine	51.24
39	L-alanyl-L-histidyl-L-isoleucyl-L-histidine	51.23
40	L-alanyl-L-alanyl-L-tyrosyl-L-methionine	51.22
41	L-alanyl-L-cysteinyl-L-histidyl-L-asparagine	51.22
42	L-alanylglycyl-L-cysteinyl-L-methionine	51.20
43	L-alanyl-L-cysteinyl-L-tyrosyl-L-lysine	51.16
44	L-alanyl-L-alanyl-L-arginyl-L-cysteine	51.15
45	L-alanyl-L-aspartyl-L-arginyl-L-lysine	51.13
46	L-seryl-L-arginyl-L-prolyl-L-histidine	51.13
47	L-alanyl-L-histidyl-L-valyl-L-proline	51.10
48	L-alanyl-L-cysteinyl-L-tyrosyl-L-methionine	51.02
49	L-alanyl-L-cysteinyl-L-seryl-L-isoleucine	50.98
50	L-seryl-L-arginyl-L-valyl-L-serine	50.96
51	L-alanylglycyl-L-phenylalanyl-L-glutamic acid	50.85

Discussion

Virtual screening is a logical extension based on searching a three-dimensional (3D) pharmacophore database or molecular aggregation, which can automatically evaluate large aggregated databases (Chen *et al*., 2009). First, pharmacophore models were created from five DPP-4 inhibitors of the statin group, which have been widely used as antidiabetics. The ten pharmacophore models produced were validated using 135 active compounds and 2500 decoy compounds.

Pharmacophore model III gave the highest area under the ROC curve (AUC) and enrichment factor (EF) values of 0.59% and 1.2, respectively. While the sensitivity (Se), specificity (Sp), accuracy (Acc), Yield of actives (Ya), and goodness of hit-list (GH) values were 0.69, 0.94, 0.84, 0.06, and 0.2, respectively. A good AUC value is 0.5 or higher, while a good EF value is 0.1 or greater. Optimal Se and Sp values are equal to one. The Ya and GH values are more desirable when higher (Braga & Andrade, 2013). This pharmacophore model showed significant potential in predicting effective molecular interactions, strengthened by high Se and Sp values, indicating good accuracy in identifying active and non-active molecules. The resulting accuracy (Acc) reflects the model's ability to correct classification, while a low Ya value indicates a limited positive balance. In the context of GH, a measure of geometric harmony, the obtained values indicate room for improvement in molecular shape matching. This model's combination of HBA, HBD, and pi-alkyl features contributes to interactions with specific biological targets, reflected by the aforementioned values. Therefore, pharmacophore model 3 was used as the virtual pharmacophore model for screening.

The virtual screening utilised the pharmacophore model 3 as the validated pharmacophore model, resulting in 51 tetrapeptides as the hits compounds with a pharmacophore fit score of more than 50.0%. This indicates that these compounds have a fairly high affinity for the pharmacophore model and are potential candidates for further drug development.

These hit compounds can be further selected based on their pharmacophore suitability scores to determine the most promising anti-diabetes drug candidates. Compounds with higher scores typically show better interactions in pharmacophore models and a greater likelihood of functioning effectively as therapeutic agents. Therefore, it is important to analyse these tetrapeptides' chemical and biological properties in depth to ensure that they match virtually any pharmacophore model and are effective and safe when tested in more complex biological systems.

Conclusion

It was concluded that the 51 tetrapeptides obtained from the pharmacophore virtual screening were potential as anti-diabetes mellitus drug candidates.

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