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Molecular Docking Investigation of *Dioscorea alata* Compounds Binding to CCKBR, CHRM3, CHRM5, and H2R for Gastric Ulcer Treatment

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Abstract

Gastric ulcer is a pathological condition characterized by damage to the mucosal and submucosal tissue layers of the stomach. The main proteins targeted in the treatment of gastric ulcers are CCKBR, CHRM3, CHRM5, and H2R because of their roles in the regulation of gastric acid secretion. *Dioscorea alata* (DA) is known to contain various active compounds with high sapogenin, diosgenin, and dioscorin content, in addition to its carbohydrate content. We investigated the potential of DA compounds to bind to these proteins *in silico*. Compound and protein structures were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov), ChemSpider (http://www.chemspider.com), and the RCSB PDB (https://rcsb.org). Proteins and compounds are prepared first, and then docked to obtain the interactions that might form, and finally visualized to observe the interactions that have been constructed. The results showed that diosgenin and prosapogenin have potential as inhibitors of CCKBR and CHRM5, and diosgenin and dimethyl batatasin IV have potential as inhibitors of CHRM3 and H2R. We conclude that diosgenin, prosapogenin, and dimethyl batatasin IV in DA compounds have the potential to inhibit CCKBR, CHRM3, CHRM5, and H2R for gastric ulcer treatment. It is necessary to further study the effect of *Dioscorea alata in vitro* through a gastric ulcer model study.

Keywords: CCKBR, CHRM3, CHRM, *Dioscorea alata*, Gastric ulcer, H2R

Introduction

The stomach is one of the main digestive organs of the gastrointestinal (GI) tract. Gastric ulcer is a pathological condition in which the mucosal and sub-mucosal tissue layers of the stomach are damaged, while peptic ulcer is a similar condition that occurs in the stomach and proximal duodenum. Despite the difference in medical terminology, the two can be caused by and result in similar physiological conditions. Gastric ulcer is characterized by nausea, loss of appetite, and vomiting. This condition can be caused by several factors, such as irregular eating patterns [1], gastric acid hypersecretion [2], NSAIDs [3], *Helicobacter pylori* (*H. pylori*) infection [4], and erosion of the gastric mucosal layer. [5,6] Gastrin is a key hormone in the regulation of gastric acid secretion. This regulation involves histamine-induced activation of the histamine H2 receptor (H2R), cholinergic muscarinic receptor 3 (CHRM3), and cholecystokinin receptor 2 (CCK2R). Gastric acid is secreted by parietal cells, which are regulated by hormonal, paracrine (histamine and somatostatin), and neuronal (vagal) factors. The gastric acid secretion process is divided into two phases, namely the cephalic and gastric phases that are mediated by the central and peripheral nervous systems and occur before food enters the GI tract. In the cephalic phase, there is an increase in gastric acid secretion mediated by acetylcholine secretion by vagal efferents, which activates CHRM3 present in parietal cells, which causes enterochromaffin-like (ECL) cells to secreted histamine. The gastric phase occurs when food enters the GI tract, where circulating gastrin interacts with CCK2R on the parietal cell membrane and induces acid secretion [2].

Dioscorea is a plant genus that is also called "yam" and has been used as a staple source of starch even before1500 M by the people of New Guinea, known as "Inhame" [7]. Dioscoreaceae is a large family of plants with around 600 species recorded [8]. They are found in areas with tropical-subtropical climates

and are used as a staple food source. Dioscorea spp. contains polysaccharides, saponins, allantoin, polyphenols, and sources of diosgenin. These plants are also often used as a traditional medicinal plant because they contain secondary compounds used in traditional Chinese medicine and Indian Ayurveda. *Dioscorea alata* (DA) is found in many tropical areas, including Indonesia. *Dioscorea alata* is known to contain a variety of compounds with high sapogenin, diosgenin, and dioscorin content in addition to its carbohydrate content. Also, DA is known to have bioactivity as an anti-inflammatory and anti-diabetic by reducing the expression of TNF-α and IFN-γ in lymphocytes and preventing mitochondrial dysfunction and insulin resistance [7,9]. However, information regarding the gastro protective bioactivity of DA in gastric ulcer treatment is still limited. We therefore explored the potential of DA compounds to inhibit CCKBR, CHRM3, CHRM5, and H2R.

Materials and methods

Protein and compound data mining

DA compounds were obtained from a previous study of 34 active compounds [9]. The 3D structures and canonical SMILES of the compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and ChemSpider (http://www.chemspider.com), which were then used for bioactivity prediction analysis. The 3D structures of the proteins were obtained from the RCSB PDB (https://rcsb.org), and the structures were evaluated for their resolution, most favored regions, G-factor score, and the 3D structure collection method using PDBSum (http://www.ebi.ac.uk/thorntonsrv/databases/pdbsum) [10].

Screening-based probable bioactivity analysis

Gastro protective bioactivity analysis was performed using the PASSOnline webserver (https://www.way2drug.com/PASSOnline) by looking at the probability to be active (Pa) score, with a higher Pa score indicating a higher probability of bioactivity. PASSOnline is a database for predicting the bioactivity of organic compounds based on the analysis of the structure-activity relationships of more than 300,000 organic compounds as a training set, which has an average accuracy of above 95 % [11]. The bioactivities selected were gastrin inhibition, gastritis treatment [12], inhibition of gastric secretion [13], and gastricsin inhibition [14].

Protein network and functional annotation analysis

Protein-protein interaction (PPI) networks were constructed using STRING (https://string-db.org/), and analyzed using Network Analyzer, and CluGO v.2.5.9. on Cytoscape v.3.9.1 [15-17]. The analysis components of the PPI included gene ontology (GO) at the level of biological process, cellular component, and molecular function, and KEGG pathways. GO provides gene annotation data from the GO consortium (GOC) and facilitates the processing of these data [18]. Kyoto Encyclopedia of Genes and Genomes (KEGG; available at https://www.kegg.jp), is an integrated and manually curated bioinformatics database that categorizes genes based on bioactivity, ontology, chemical processes, and health information. KEGG pathways can be used to analyze gene interactions and display them in taxonomic form. KEGG pathways can provide information such as pathways that are induced, related genes, and physiological effects [19].

Molecular docking analysis

Molecular docking analysis was carried out on 10 selected active compounds that had the highest average bioactivity scores, as well as the diosgenin compound as the fingerprint compound of DA. The grid coordinates used on Vina in PyRx 0.9.7 were as follows: CCKBR, center X: 113.268, Y: 114.722, Z: 140.207; Dimension (Angstrom) X: 32.414, Y: 30.552, Z: 38.089. CHRM3, center X: 44.114, Y: 94.400, Z: 55.418; Dimension (Angstrom) X: 18.948, Y: 17.394, Z: 21.744. CHRM5, center X: 34.412, Y: 21.964, Z: −47.861; Dimension (Angstrom) X: 26.621, Y: 32.605, Z: 45.845. H2R, center X: 160.052, Y: 166.197, Z: 198.254; Dimension (Angstrom) X: 22.398, Y: 22.119, Z: 21.143. Energy minimization of proteins was performed using Swiss PDBViewer, and the proteins were prepared into a macromolecular auto dock format and then docked with active compounds that had been prepared using OpenBabel. Ligand-protein interactions were visualized in 3D and 2D using Biovia Discovery Studio v. 19.1 [20-22].

Results and discussion

Bioactivity prediction

DA compounds were analyzed using PASSOnline with the bioactivity categories gastrin inhibitors, gastritis treatment, gastric anti-secretory, and gastricsin inhibitors. P-coumaric acid, dihydropinosylvin, and ferulic acid had the highest average probability to be active (Pa) scores (**Table 1**).

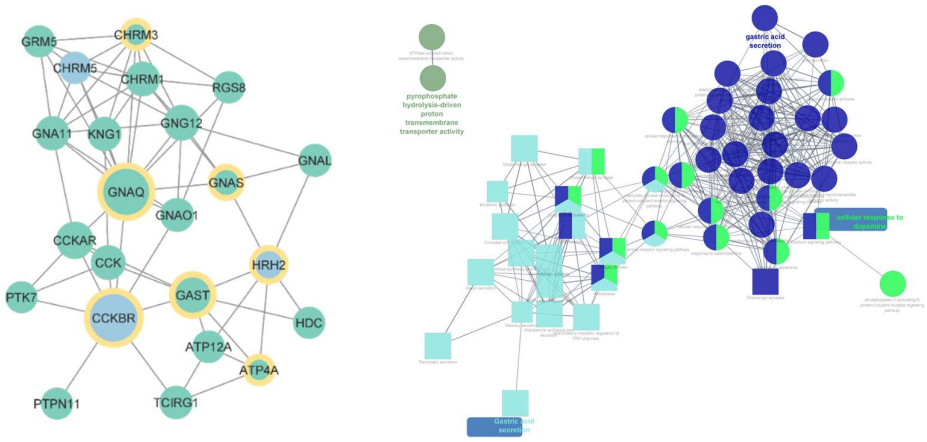
Table 1 Bioactivity prediction from PASSOnline. The numbers in bold are the highest bioactivity prediction scores in each category.

No	Compound	Gastrin inhibitor	Gastritis treatment	Gastric antisecretory	Gastricsin inhibitor
1	allantoin	0.563	0.000	0.165	0.000
2	ascorbic acid	0.000	0.213	0.000	0.000
3	batatasin I	0.337	0.310	0.000	0.000
4	batatasin II	0.548	0.293	0.000	0.000
5	batatasin III	0.591	0.306	0.229	0.000
6	batatasin IV	0.553	0.300	0.188	0.000
7	catechin	0.272	0.275	0.169	0.000
8	chalcone naringenin	0.499	**0.429**	0.196	0.000
9	chlorogenic acid	0.000	0.271	0.000	0.000
10	choline	0.000	0.000	0.000	0.000
11	cinnamic acid	0.000	0.000	0.000	0.000
12	cyanidin	0.000	0.000	0.000	0.000
13	cyanidin-3-O-glucoside	0.000	0.000	0.000	0.000
14	dimethylbatatasin IV	0.565	0.288	0.175	0.073
15	dihydrokaempferol	0.491	0.290	0.000	0.000
16	dihydropinosylvin	0.606	0.289	0.218	0.088
17	dihydro quercetin	0.401	0.312	0.000	0.000
18	diosbulbin B	0.000	0.000	0.000	0.000
19	diosgenin	0.000	0.000	0.000	0.000
20	ferulic acid	0.574	0.384	0.224	0.000
21	gracillin	0.423	0.000	0.000	0.000
22	leucocyanidin	0.271	0.274	0.216	0.000
23	leucopelargonidin	0.378	0.254	0.239	0.000
24	mucic acid	0.614	0.255	0.000	**0.124**
25	myricetin	0.000	0.354	0.000	0.000
26	naringenin	0.649	0.280	0.000	0.000
27	nicotinamide	0.411	0.159	**0.259**	0.000
28	p-coumaric acid	0.585	0.344	0.208	0.076
29	pelargonidin	0.000	0.000	0.000	0.000
30	peonidin	0.000	0.000	0.000	0.000
31	prosapogenin	**0.701**	0.000	0.000	0.000

No	Compound	Gastrin inhibitor	Gastritis treatment	Gastric antisecretory	Gastricsin inhibitor
32	sapogenin	0.000	0.000	0.000	0.000
33	γ-sitosterol	0.406	0.000	0.000	0.000

Protein-protein network and functional annotation analysis

Pathway analysis of the H2R, CCKBR, and CHRM5 proteins indicate several proteins that play a role in the gastric acid secretion pathway based on gene ontology and KEGG pathway analysis. The results of the protein enrichment network also show other genes related to gastric acid secretion bioactivity, namely CHRM5, GNAQ, GNAS, GAST, and ATP4A. Knowing these interacting proteins can provide more insight into possible protein targets associated with gastric acid secretion. The bioactivity of gastric acid secretion in GO and KEGG is known to be connected with the cellular response to dopamine, wherein dopamine signaling via dopamine receptors (DARs) and/or androgenic receptors can influence gastrointestinal (GI) motility, absorption, secretion, and protection of GI mucosa. (**Figure 1**, **Table 2**).



(A) (B)

Figure 1 Protein-protein interaction network (PPI) and predicted bioactivity results based on gene ontology (GO) and KEGG enrichment. (A) PPI network. Proteins colored blue are annotated with the GO term Gastric acid secretion (GO: 0001696). Proteins highlighted with a yellow ring are annotated with the KEGG pathway term gastric acid secretion (hsa04971). The network was analyzed using STRING (https://string-db.org/). (B) Bioactivity network resulting from enrichment of biological process GO terms (circles) and KEGG pathway enrichment (squares).

Table 2 Protein network functional enrichment.

No	ID	Bioactivity	Term *p*-value	Corrected term *p*-value	Gene
1	GO:0001696	Gastric acid secretion	1.92e-06	3.46e-5	ATP4A, CCKBR, CHRM3, GAST, GNAQ, GNAS, HRH2
2	KEGG:04971	Gastric acid secretion	3.61e-12	1.41e-10	CCKBR, CHRM5, HRH2

Molecular docking result

Receptor protein evaluation in molecular docking analysis was carried out to select the protein with the best macromolecular structure based on the resolution, most favored region, and G-factor score available in the PDB. In this evaluation, we chose the protein with the highest resolution that had the most favored region of more than 85% and a G-factor score above −0.05, with the hope that the protein used in this study would approach normal and actual conditions in the body. Resolution is a scale that shows the clarity of the diffraction pattern of a structure, which can be seen from the electron density map. The high-

resolution structure (around 1 Å) has atoms that are very clear to observe through the electron density map, while the lower-resolution structure (more than 3 Å) has atoms that are more difficult to observe. The most favored region and G-factor score are calculated values based on PROCHECK analysis. PROCHECK analysis is carried out by assessing the geometry scores and regions of each protein that allow for "unusual" or wrong structures to occur. Protein structures with good quality have at least a percentage of the most favored region of 90%, and protein structures that are "unusual", or out-of-the-ordinary, have a G-factor score below −0.05 [23,24]. Molecular docking analysis was performed on 4 proteins (CCKBR, CHRM3, CHRM5, and H2R) as receptors and 11 ligands consisting of a drug control, 5 ligands with the highest average bioactivity prediction score, 4 ligands with the highest predictive score for each bioactivity, and the active compound diosgenin, which is known to be a fingerprint compound of DA (**Table 3**).

Based on the results of molecular docking analysis, the DA compounds with the highest predicted affinity for CHRM3, CHRM5, CCKBR, and H2R were dimethyl batatasin IV, prosapogenin, and diosgenin. The comparison of interactions between DA compounds and drugs shows that prosapogenin and diosgenin have a higher affinity for interacting with CCKBR and H2R, respectively. Based on the docking results on CCKBR, pentagastrin, prosapogenin, and diosgenin have overlapping docking sites. As a result, there are amino acids Pro114, Met134, Val138, His207, and His367 that can form hydrophobic interactions with the 3 ligands. The result of the DA compound interaction in CHRM3 is formed in a similar location to the drug that also forms hydrophobic interactions with an alanine residue (Ala238). Tiotropium forms 4 hydrogen bonds and 2 electrostatic interactions with CHRM3. In CHRM5, the tiotropium docking site is parallel to the prosapogenin site and perpendicular to the diosgenin site. The different docking locations mean that the exact residues that interact with the 3 ligands are different. Subsequent docking results for H2R show that famotidine and dimethyl batatasin IV have docking sites that overlap each other, while diosgenin has a docking site that is parallel to these sites. Based on the docking location, it was observed that famotidine and dimethyl batatasin IV can form hydrogen bond with Val176 in H2R, while diosgenin forms interactions with amino acid residues that are different to the other two ligands (**Figure 2**, **Tables 4** - **5**).

In this study, the potency of the DA compounds as inhibitors of the 4 proteins was predicted from the binding energy, the binding pose, and the interacting amino acid residues compared to the control drug. Prosapogenin and diosgenin have high potential to serve as CCKBR inhibitors because they are predicted to be able to compete with pentagastrin (gastrin-like molecule). Dimethyl batatasin IV has potential to serve as a CHRM3 inhibitor because its binding energy is the lowest after tiotropium (a CHRM3 inhibitor drug), whereas prosapogenin and batatasin III have little potential to serve as CHRM5 inhibitors. Also, diosgenin and demethyl batatasin IV require a lower binding energy on H2R compared to the binding energy of famotidine on H2R. Therefore, both have potential as H2R inhibitors because of their good affinity.

Table 3 Protein structure evaluation.

No	Protein (Gene)	PDB ID	Resolution (Å)	Drug	Method	Most favored regions (%)	G-factor score
1	CCK2R (*CCKBR*)	7F8W [25]	3.10	Pentagastrin (DB: 00183)	EM	86.3	0.02
2	mAChR3 (*CHRM3*)	4U15 [23]	2.80	Tiotropium (DB: 01409)	X-ray	92.3	0.37
3	mAChR5 (*CHRM5*)	6OL9 [24]	2.54	Tiotropium (DB: 01409)	X-ray	93.0	0.29
4	H2R (*HRH2*)	7UL3 [26]	3.00	Famotidine (DB: 00927)	EM	93.5	0.20

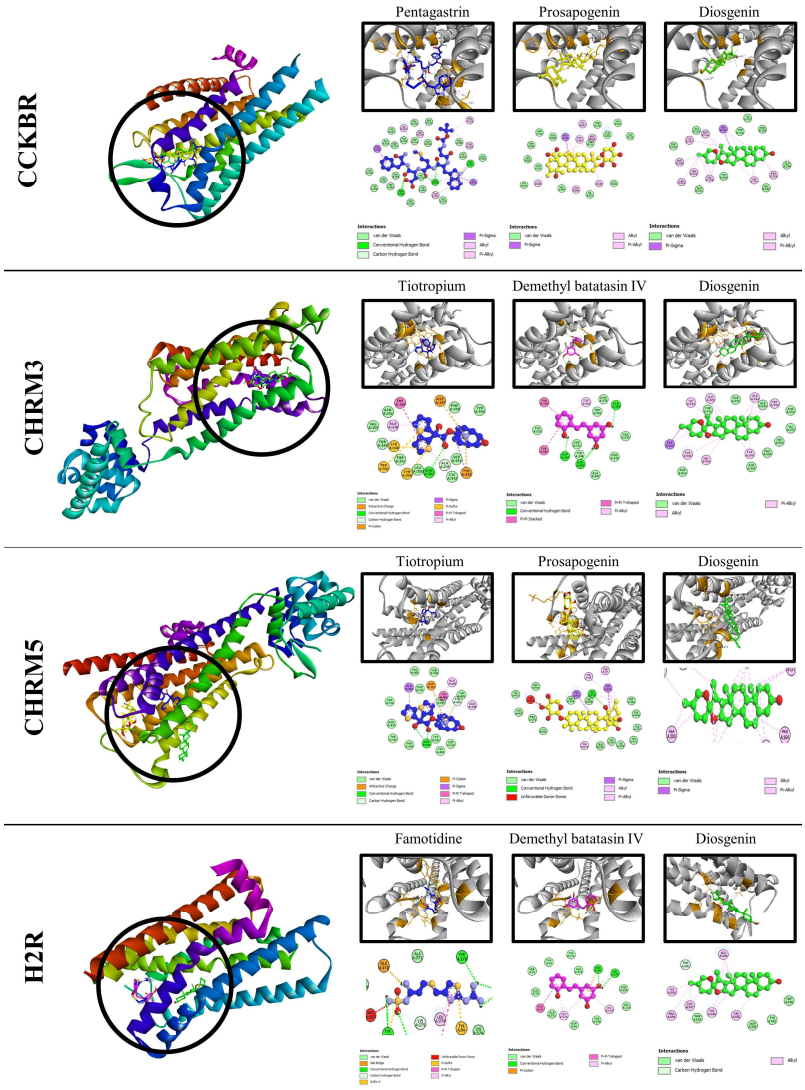


Figure 2 Docking positions of ligands and interacting amino acids.

Table 4 Binding affinity result (kcal/mol). The numbers in bold indicate the lowest binding energy value of DA compounds.

No	Ligand	ID	CCKBR	CHRM3	CHRM5	H2R
1	control		−9.5	−9.3	−9.9	−6.8
2	diosgenin	CID: 99474	−9.0	−3	−8.8	**−8.6**
3	batatasin III	CID: 10466989	−7.6	−8.1	−9.0	−7.2
4	demethyl batatasin IV	CID: 442699	−7.5	**−8.6**	−8.8	−7.2
5	dihydro-pinosylvin	CID: 442700	−7.2	−8.5	−8.6	−7.2
6	ferulic acid	CID: 445858	−5.9	−6.9	−7.0	−6.2
7	mucic acid	CSID: 2301286	−4.7	−5.3	−5.4	−5.8
8	naringenin chalcone	CID: 5280960	−7.0	−8.4	−8.0	−6.8
9	nicotinamide	CID: 936	−4.9	−5.3	−5.3	−6.2
10	p-coumaric acid	CID: 637542	−5.7	−6.6	−7.1	−6.0
11	prosapogenin	CSID: 10252038	**−9.6**	−3.6	**−9.4**	−5.3

Table 5 Residues that interact with ligands. Residues printed in bold are those that interact with the control drug.

No	Protein	Ligand	Hydrogen bond	Hydrophobic interaction	Electrostatic interaction	Others
1	CCKBR	Pentagastrin (control)	Asn353 (3), Arg356 (4), Leu367	Pro114, Phe120, Met134, Val138 (2), His207, Ala352, Arg356, Leu367 (2), Ile372 (2),	-	-
2	CCKBR	Prosapogenin	-	**Pro114**, **Phe120**, **Met134**, **Val138** (2), **His207**, Trp346, **His367** (3),	-	-
3	CCKBR	Diosgenin	-	Cys107, **Pro114**, **Met134** (2), **Val138** (3), Met186, **His207** (2), Leu222, Leu226, **His367** (3),	-	-
4	CHRM3	Tiotropium (control)	Asp147 (2), Ala235, Asn507	Trp199, Ala235, Ala238, Tyr529	Asp147, Tyr529	Tyr148 (Pi-Sulfur), Trp503 (Pi-Sulfur), Tyr506 (Pi-Sulfur)
5	CHRM3	Demethyl-batatasin IV	Asp148, Ala238, **Asn507** (2)	**Ala238**, Tyr506, **Tyr529**, Cys532	-	-
6	CHRM3	Diosgenin	-	Tyr148 (2), **Trp199** (3), Leu225 (4), **Ala238** (3), Tyr506, Val510, Trp525 (3),	-	-
7	CHRM5	Tiotropium (control)	Asp110, Asn459 (2), Tyr481	Leu188, Ala198, Ala201, Tyr458, Tyr481	Asp110, Tyr481	-
8	CHRM5	Prosapogenin	His478	Tyr87, Tyr90, Val474, Trp477 (2), His478, **Tyr481** (2),		Lys470 (uf)
9	CHRM5	Diosgenin	-	Pro164, Ala165 (4), Trp169 (2), Leu172 (2), Phe187 (3), Ile193, Ala200 (2), Ala201	-	-
10	H2R	Famotidine (control)	Asp98 (2), Lys175, Val176, Tyr250 (2), Glu270, Tyr278,	Tyr78, Leu274	-	Tyr94 (Sulfur-X) Tyr94 (P i-Sulfur) Tyr250 (Pi-Sulfur) Arg257 (uf)
11	H2R	Demethyl-batatasin IV	**Val176**, Arg257	**Tyr78**, Lys175, Val176, **Leu274** (2)	Arg257	-
12	H2R	Diosgenin	Thr190	Ile106, Leu107 (2), Leu149 (3), Val185, Val189 (2), Pro194	-	-

Discussion

DA is a species in the genus Dioscorea that has been domesticated in Asia and is cultivated globally. Apart from being a source of starch, DA contains other secondary compounds, including saponins, alkaloids, flavonoids, and a small number of tannins and phenols. The saponin content can be as high as 2.98 in 100 mg of dry tuber extract [7]. Saponins are compounds that are known to have various bioactivities, including fungicidal, anti-viral, anti-microbial, anti-cancer, anti-inflammatory, and immunomodulatory activities. Saponins are amphiphilic molecules consisting of carbohydrates and triterpenoid or steroid aglycone groups. DA is known to contain saponin compounds including diosgenin (3-β-hydroxy-5spirostene), prosapogenin, and gracillin [9, 27]. In this study, diosgenin was predicted to interact with all 4 receptors, and prosapogenin was predicted to have a low binding energy for CCKBR and ACM5.

Diosgenin is a furostanol saponin, known to have various bioactivities, and has been developed as a starting material in the pharmaceutical industry for anti-inflammatory, estrogenic, and androgenic steroid drugs. Diosgenin is known to reduce the production of pro-inflammatory cytokines such as interleukins 1, interleukin 6, and nitric oxide (a cytokine-like molecule), as well as blocking CK2 activation and phosphorylation of c-Jun NH(2)-terminal kinase (JNK) [28–30]. Prosapogenin A (PSA; (3β,20β)-19-Hydroxy-3-(β-D-xylopyranosyloxy)urs-12-en-28-oic acid) is a steroidal saponin found in the genus Discorea, including DA. PSA was also reported to be found in D. zingiberensis, which has been used for a long time in traditional Chinese medicine. *In vitro*, it is known that PSA can induce apoptosis of the HeLa, HepG2, and MCF-7 cancer cell lines through inhibition of the STAT3 pathway, and induce expression of glycometabolism-related genes [31, 32]. Other studies have shown that PSA can induce caspase 3/7

activation, increase ROS production, and down regulation of MMP (matrix metalloproteinase), which results in the induction of apoptosis in CCRF-CEM cells [33].

Dimethyl batatasin IV (5-[2-(2-hydroxyphenyl)ethyl]benzene-1,3-diol) is a phytoalexin derived from batatasin, which is a group of phenolic compounds first isolated from dormant yam bulbils and known to inhibit their budding [34]. Dimethyl batatasin IV was also reported to be found in D. bulbifera from China and Nigeria from tuber and bulbil extracts [35]. Information on the pharmacological effects of these compounds is still very limited, with one study reporting that dimethyl batatasin IV can interact with human serum albumin with a strong association constant [36]. In this study, we report that dimethyl batatasin IV was predicted to bind to all 4 proteins with low binding energy.

Gastritis patients may experience fatigue, stomach pain, nausea, vomiting, loss of appetite, pale skin, and, worst of all, vomiting blood [1,37]. Practically identical to gastritis, gastric ulcer, is a condition in which the mucosa is eroded to the submucosa, or muscular mucosa, in the stomach or proximal duodenum. This disease is estimated to occur in 8.09 million of the world's population in 2019 [38]. Not only has there been a decrease in new cases, but also an increase in the age-standardized prevalence rate from 1990 until 2019 (from 143 to 99 years old). Gastric ulcers have various causes, but use of NSAIDs and infection with *H. pylori*are have been reported to be the most prevalent [3, 4, 38]. Several treatments have been developed, including H2R antagonists and proton pump inhibitors (PPIs), to reduce the effects and secretion of gastric acid [4]. The secretory phase of gastric acid begins even before food enters the GI tract since it is stimulated by the nervous system. Gastric acid secretion is regulated by various signaling and hormonal factors to maintain homeostasis. Several proteins play a role in gastric acid secretion, including H2R, CCKBR, CHRM3, CHRM5, GNAQ, GNAS, GAST, and ATP4A. Current gastric ulcer treatments target the H2R and CCKBR proteins. In addition, targeting mAChRs is also interesting to investigate considering that this receptor is found in parietal cells in the stomach, while targeting GNAQ, GNAS, GAST, and ATP4A for gastric ulcer treatment is a topic of ongoing research [2, 39].

Famotidine is a selective H2R antagonist that can inhibit gastric acid secretion 20 - 50 times better than cimetidine in patients with gastric acid hypersecretory disease [40]. In addition, famotidine and cetirizine, which are H2R and H1R blockers used as anti-inflammatory drugs in COVID-19 patients, can reduce the likelihood of death and the severity of symptoms in COVID-19 sufferers [41]. Based on the results of this study, famotidine can form 8 hydrogen bonds in H2R, one of which, at Val176, is also formed in the interaction of dimethyl batatasin IV with the peptide backbone, which had a higher predicted binding energy than the other DA compounds.

Tiotropium is a muscarinic antagonist developed to treat chronic obstructive pulmonary disease (COPD),which can bind with high affinity to a variety of muscarinic receptors, including M1 (mAChR1), M2, and M3 [42]. In this study, tiotropium was predicted to bind with high affinity to M5, had higher affinity than any of the DA compounds, and formed comparable hydrogen bonds and electrostatic interactions in both receptors (CHRM3 and CHRM5). Other studies have shown that tiotropium can reduce the number of eosinophils in bronchoalveolar lavage fluids, and reduce IL-4, IL-5, IL-13, and group 2 innate lymphoid cells (ILC2s) *in vivo* [43]. Dimethyl batatasin IV and prosapogenin were the DA compounds with the lowest predicted binding energies to the muscarinic receptors.

Pentagastrin is a gastrin-like molecule that was developed to evaluate and examine the function and secretion of gastric acid, gastric acid hypersecretion, and Zollinger-Ellison tumors. The use of pentagastrin is also known as the gastrin test, in which 6 µg of pentagastrin is used for each kg of the subject's body weight. The use of this test shows a minor side-effect and has the advantages of being short, and simple, as well as having reliable results [44]. In our study, pentagastrin formed 8 hydrogen bonds and 12 hydrophobic interactions with CCKBR, and was predicted to bind with high affinity. As for the DA compounds, prosapogenin had the lowest binding energy and even lower binding energy than pentagastrin, while diosgenin had the second lowest binding energy and formed more hydrophobic interactions than pentagastrin. Overall, our study suggests that diosgenin, prosapogenin, and dimethyl batatasin IV in DA compounds have the potential to act as inhibitors of CCKBR, CHRM3, CHRM5, and H2R, forming the basis for further gastric ulcer treatment research.

Conclusions

Several DA compounds, including dimethyl batatasin IV, prosapogenin, and diosgenin were predicted to bind to CCKBR, CHRM3, CHRM5, and H2R for gastric ulcer treatment, and should be tested in future for inhibitory activity. It is necessary to further study the effect of *Dioscorea alata in vitro* on gastric primary cell culture system.

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