

## Molecular interaction between xanthorrhizol with ghrelin-o-acyl transferase (GOAT) and growth hormone SECRETAGOG3UE receptor (GHS-R): A docking analysis

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### Abstract

After curcuminoids, xanthorrhizol is the second primary bioactive compound from *Curcuma xanthorrhiza*. Traditionally, the rhizome of *Curcuma xanthorrhiza* is well-known for its appetite stimulation. However, the mechanism of appetite stimulation is still unclear, particularly its interaction with the appetite-related ghrelin system. Therefore, understanding interactive molecular modulations of xanthorrhizol with ghrelin, GOAT (ghrelin-O-acyl transferase), and ghrelin receptor (GHS-R: growth hormone secretagogue receptor) is essential. Xanthorrhizol, an appetite stimulant, may interact with ghrelin, GOAT, and GHS-R. Herein, this study aimed to do in silico analysis to hypothetically predict the molecular interaction of xanthorrhizol with ghrelin, ghrelin-O-acyl transferase (GOAT), and growth hormone secretagogue receptor (GHS-R). Docking analysis was conducted to understand the interactive molecular patterns of xanthorrhizol, ghrelin, GOAT, and GHS-R. The docking studies showed that the molecular interaction of xanthorrhizol is with Arg10 of ghrelin and not with Ser2, which is essential for the linkage of octanoic acid. The molecular interaction of xanthorrhizol with GOAT is in the active sites of either deacylated ghrelin or octanoic acid. Therefore, xanthorrhizol is a competitive inhibitor of GOAT. It inhibits GOAT activity. Xanthorrhizol interacts with GHS-R in its small cavity II. It is still unclear whether this interaction is agonist or antagonist. This in silico analysis showed that xanthorrhizol is probably not a stimulating appetite natural drug via stimulating GHS-R signaling.

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### Keywords

Appetite stimulant, *Curcuma xanthorrhiza*, enzyme modulation, hunger hormone, temulawak

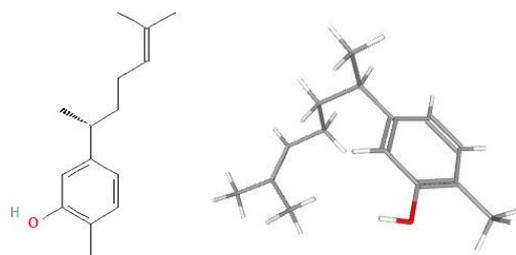
## 1. Introduction

Xanthorrhizol (XNT) (2-methyl-5-[(2R)-6-methylhept-5-en-2-yl] phenol) is a bisabolane-type sesquiterpenoid (Fig. 1). It can be extracted from the rhizome of *Curcuma xanthorrhiza* (local name: temulawak). The rhizome is an essential component of Indonesian traditional medicine (local name: Jamu) and is extensively utilized as a medicinal and nutritional plant. Traditionally, it is used to treat diseases like lack of appetite, children's fever, stomach and liver disorders, constipation, bloody diarrhea,

dysentery, arthritis, hypotriglyceridaemia [1], hemorrhoids, and rheumatism [2]. It also has various bioactivities, like antioxidant, anti-inflammatory, anticancer, antidiabetic, antihypertensive, antiplatelet, antimicrobial, skincare, and nephron-hepatoprotective properties [1-4].

Our body has appetite-related hormones (orexigenic hormones), like ghrelin. As an orexigenic hormone, ghrelin (acylated ghrelin, AG) regulates homeostatic and reward-related feeding behavior. This acylated





**Figure 1.** Chemical structure of Xanthorrhizol (PubChem)

ghrelin comes from the acylation of ghrelin from desacyl ghrelin (DAG). Ghrelin O-acyl transferase (GOAT), a gut enzyme, catalyzes the acylation of DAG [5]. In most cases, AG carries the octanoyl group in its third amino acid, serine (Ser2) [6]. Ghrelin impacts hunger and metabolic regulation by binding the ghrelin receptor (GHS-R) for signal activation [7]. By signal activation of GHS-R, GHS-R can regulate energy homeostasis and body weight. Ghrelin activation to GHS-R can directly stimulate appetite and hunger signaling [7]. GHS-Rs are most highly expressed in the hypothalamus, distinctively the ventromedial nucleus and arcuate nuclei. However, expression of GHS-Rs also happens in other areas of the brain, including the hippocampus, substantia nigra, and ventral tegmental area. Outside the central nervous system, GHS-Rs also exist in the heart, liver, and skeletal muscle [6].

To understand the relationship between ghrelin and appetite-related behavior, we must know the structures and functions of ghrelin (AG), DAG, GOAT, and GHS-R. Together is recognized as a Ghrelin/GOAT/GHS-R1a system (G3S). Specifically, G3S is essential in energy homeostasis that signals appetite and hunger [7].

## 2. Materials and methods

Can XNZ improve appetite behavior by interacting with Ghrelin, GOAT, and GHS-R? What are the molecular interactions between XNZ with Ghrelin, GOAT, and GHS-R? Can XNZ influence the activity of GOAT and GHS-R signaling? and finally, can XNZ modulate appetite behavior through its Interaction with GOAT and GHS-R?

We hypothesized that the XNT has molecular interaction with Ghrelin and GOAT. In addition, XNZ has strong interaction in the active or allosteric sites of GOAT and GHS-R. Moreover, XNZ is an agonist of GHS-R.

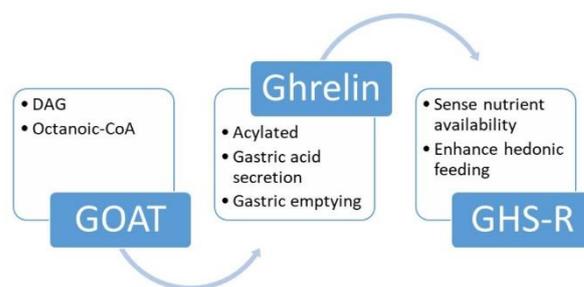
Receptors	: Desacyl Ghrelin (DAG), GOAT and GHS-R
Endogenous ligands of GOAT	: Desacyl Ghrelin (DAG) and o-octanoic acid
Endogenous ligand of GHS-R	: Ghrelin (acyl ghrelin, AG)
Exogenous ligands	: Xanthorrhizol

## 3. Results and Discussion

### 3.1. Octanoylation/Acylation process by GOAT

#### 3.1.1. General characteristics of ghrelin

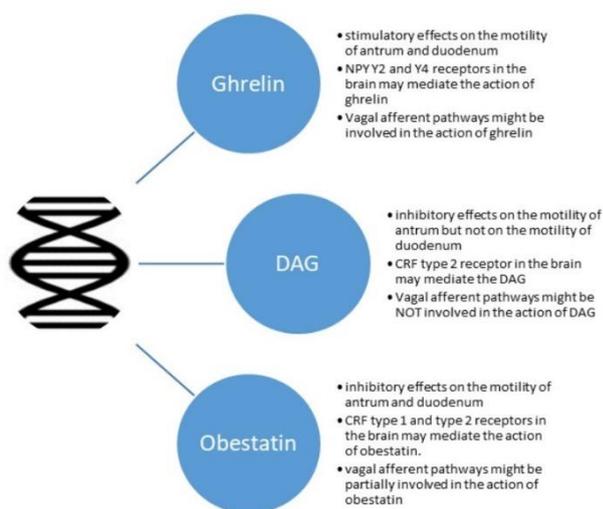
Ghrelin is an unusual peptide stomach hormone that is consisted of 28 amino acid residues. Its desacyl form, DAG, undergoes acylation or octanoylation of its third amino acid or Ser2. This acylated ghrelin (AG) is essential for ghrelin's activity to signal its receptor, named GHS-R (Fig. 2). [8] GOAT catalyzes DAG octanoylation [7]. The produced ghrelin acts as an endogenous ligand of GHS-R [8]. Therefore, DAG's acylation or octanoylation is essential for releasing ghrelin-induced growth hormone from the pituitary that stimulates appetite [9]. Ghrelin is considered the only peripheral hormone to transmit satiety or appetite signals. Nevertheless, ghrelin has additional physiological functions, like the stimulation of growth hormone release and accumulation of fat (obesity) (Fig. 2) [8, 10].



**Figure 2.** The function of ghrelin as a controller of homeostatic and hedonic feeding

Ghrelin is one of three hormone peptides encoded by the same preproghrelin gene. The other two hormone peptides are DAG and obestatin (Fig. 3). They modulate appetite, adipogenesis, glucose metabolism, immunity, sleep, anxiety, stress, and regulation of feeding-stimulated gastroduodenal motility. The stomach may regulate gastrointestinal motility via AG, DAG, and obestatin [11]. Even ghrelin is produced in the stomach, but its activities exert in the central nervous system by crossing the BBB. The produced

ghrelin can stimulate the secretion of growth hormone (GH).



**Figure 3.** Three hormone peptides from a single gene

Therefore, ghrelin is thought to directly affect neurons involved in feeding via GH secretion by activated GHS-R (ghrelin receptor).

Several gastrointestinal hormones, including ghreline, can transmit signals to the brain via the vagal afferent system. Vagotomy abolishes or attenuates GH secretion and the ghrelin's action on feeding. The vagal afferent system can convey the ghrelin's signals for feeding and GH secretion to the brain [12]. Blood-brain barrier (BBB) controls the entry of ghrelin, into the brain. Once ghrelin is present in the brain, it can activate the hypothalamus for regulating food intake, in the hippocampus for regulating neurogenesis, and in the olfactory bulb for regulating food-seeking behavior [13].

A preproghrelin gene encodes three peptides, namely ghrelin (or acyl ghrelin; AG), des-acyl ghrelin (DAG), and obestatin. Although DAG is considered as a degradation product of AG, DAG is considered as a separate hormone that has its own receptor and also can interact with AG at its receptor. Actually, DAG is a functional inhibitor of AG [14].

AG, DAG, and obestatin are both active hormones [15]. They are derived from a common prohormone, preproghrelin [16]. Ghrelin has orexigenic, but DAG and obestatin have anorexigenic properties. Ghrelin is produced mainly in the stomach and is an endogenous ligand of GHS-R located in the brain. The ghrelin levels in plasma strictly depend on recent food intake. Therefore, it is essential in appetite and meal

initiation [17]. While ghrelin activates GHS-R, DAG does not [18]. Ghrelin can activate the pituitary and hypothalamus in stimulating appetite and adiposity and releasing growth hormone through its activation of GHS-R-1a. However, DAG, the unacylated ghrelin form, does not bind GHS-R-1a and is devoid of endocrine activity. But in plasma, DAG is more abundant than ghrelin [19].

### 3.1.2. General characteristic of Ghrelin O-acyltransferase (GOAT)

The only peptide known to undergo octanoylation is ghrelin. This octanoylation is catalyzed by ghrelin O-acyltransferase (GOAT). GOAT is able to attach octanoate to DAG, and then produce AG, [20]. GOAT is expressed mainly in the gastrointestinal (GI) tract [21], is secreted by stomach X/A-like cells, and plays a role in appetite and metabolism [22]. DAG in the blood can cross BBB but it cannot bind to GHS-R1a. AG, but not DAG, can upregulate the GOAT expression [23]. The presence of GOAT in the hippocampus is essential for acylating DAG locally. The expression of GHS-R1a may be related to the synthesis of GOAT in the hippocampus [24].

### 3.1.3. Octanoylation process

GOAT is the only recognized enzyme that can catalyze the acyl modification of DAG that results in acylated ghrelin (AG). GOAT modifies the third amino acid serine (Ser2), not the other DAG peptides' residues. DAG and n-octanoic acids are substrates and ligands, respectively, for GOAT [25]. Octanoyl acyl donor should be supplied externally. Additionally, a four-amino acid peptide derived from the N-terminal sequence of ghrelin constitutes the core motif for substrate recognition by GOAT [26]. GOAT esterifies an n-octanoic acid to DAG, resulting in acylated ghrelin (AG) that can bind and activate the GHS-R (Fig. 2) [27, 28].

Ghrelin has a vital role in regulating glucose metabolism. GOAT can modify ghrelin into its active form [6, 29-31]. Its activity is associated with hedonic feeding behavior that is mediated by forebrain orexin signaling. The GOAT-ghrelin system is essential in mediating food motivation and hedonic feeding [5].

Activation or inhibition of GOAT depends on the physiologic situation. The fasting and satiation conditions can activate GOAT. For the GOAT's activity, octanoic acid is needed as its substrate.

GOAT can use octanoic acid either from diet-derived or adipose-fatty acids. Dietary fatty acids are probably a primary source of octanoate available in the stomach. However, there is a possibility of endogenous production of octanoate in the GOAT-expressing cells.

Moreover, the white adipose tissue can release fatty acids for GOAT to activate ghrelin, particularly during fasting. This situation is consistent with circulating ghrelin levels that increase during food deprivation. Long-term fasting can inhibit acylation but not the secretion of ghrelin. This situation is correlated with the ghrelin level that increases before meals and decreases after meals [32].

Inhibitors of GOAT can indirectly decrease ghrelin levels [33]. Specific GOAT inhibitors of GOAT can block an octanoyl attachment to ghrelin. GOAT is subjected to end-product inhibition [21]. There are two groups of GOAT inhibitors: ghrelin peptide-mimetic and small-molecule inhibitors (non-peptide-based GOAT inhibitors). An example of a ghrelin mimetic inhibitor is GO-CoA-Tat, a kind of peptide that antagonizes GOAT [30, 34]. GO-CoA-Tat attenuates AG production and prevents weight gain. In addition, GO-CoA-Tat can also increase glucose-induced insulin secretion. Therefore, inhibition of GOAT is an alternative strategy for treating obesity and related metabolic disorders [35]

Small-molecule GOAT inhibitors, like triterpenoid GOAT inhibitors, compound A and B. Synthetic triterpenoids are discovered and identified as CDDO (2-cyano-2,12-dioxoleane-1,9(11)-dien-28-oic acid). Compound A (2-[(2,4-dichlorobenzyl) sulfanyl]-1,3-benzoxazole-5-carboxylic acid) and compound B (4-chloro-6-(1-benzothiophen-3-yl) acetic acid) can be synthesized and inhibit GOAT. They show octanoyl-CoA competitive inhibitory activity and can decrease acyl ghrelin production [22].

Ghrelin is a potent food intake stimulator, leading to weight gain and adiposity. It can increase the risk of obesity and binge eating behavior. The functionality of ghrelin is due to its interaction with the GHS-R1a. Besides its ability to promote the reinforcement of hedonic food, it also acts at extra-hypothalamic sites, making interaction with dopaminergic, cannabinoid, opioid, and orexin signaling [36].

3.2. *Growth hormone secretagogue receptor (GHS-R) (ghrelin receptor)*

### 3.2.1. *General characteristics of GHS-R*

GHS-R belongs to the G-protein-coupled receptors (GPCRs) that mediate extracellular to intracellular signaling for various physiological functions. GPCRs form binding with orthosteric or allosteric ligands that modulate their activity [37]. GHS-R, as the ghrelin receptor, mediates various biological effects of ghrelin. Activation of GHS-R may trigger a diversity of signaling mechanisms and physiological responses. Information on the molecular structure of GHS-R, ligand-receptor interaction, and its intracellular signaling pathways is essential for understanding the interaction of XNT and GHS-R [38].

Two forms of ghrelin, AG and DAG, are primarily present in the plasma with GOAT. DAG has antagonist properties and can counteract the effects of AG. AG and DAG can influence the hypothalamic-pituitary-adrenal (HPA) axis and the corticosterone/cortisol level that drives the eating desire under stressful situations. DAG and inhibition of GOAT are good targets for reducing obesity and bingeing-related eating disorders. Furthermore, AG/DAG ratio is an essential biomarker for diagnosing maladaptive eating behaviors [36]. As a ligand of GHS-R, ghrelin is considered a short-term meal initiator and a long-term energy balance regulator. AG protein-coupled receptor is identified in the human pituitary and hypothalamus, stimulating the GH release from the anterior pituitary.

### 3.2.2. *Signaling mechanism of GHS-R*

There are two GHS-R transcripts, GHS-R1a, and GHS-R1b. GHS-R1a is the acyl ghrelin receptor that is expressed in the brain and other body areas. Multiple GHS-R1a agonists, antagonists, and inverted agonists are available [39]. GHS-R1a can be expressed in the hypothalamus's feeding or appetite-regulating center [24]. AG is a ligand for GHS-R1a, and acts on GHS-R1a to stimulate GH release. The GHS-R1a is essential in eating behavior and the pathogenetic mechanisms of drug addiction, obesity, and chronic alcohol consumption [40].

Octanoylated ghrelin (AG) is able to activate GHS-R1a, and is involved in multiple physiological processes, including stimulus of food intake, gastric exhausting, body energy balance, glucose homeostasis, reduced

secretion of insulin, and lipogenesis. There are several GHS-R1a ligands. They are peptidyl and non-peptidyl ligands that act as GHS-R1a agonists, antagonists, or inverse agonists [41]. With their interaction, GHS-R1a mediates the pharmacological properties of ghrelin [42]. As a ligand of GHS-R, ghrelin may bind to GHS-R after its acylation or octanoylation on its serine-3-residue by GOAT. Therefore, the administration of ghrelin increases food intake and body weight. On the contrary, inhibiting its actions with GHS-R leads to decreased food intake and weight loss.

Ghrelin acts as an agonist at the ghrelin receptor because it modulates its maximum efficacy and potency [43, 44]. Ghrelin is a hunger hormone that can activate GHS-R, stimulate food intake and growth hormone secretion, and regulate reward signaling. Therefore, ghrelin can promote body weight gain and adipogenesis. Acylation of ghrelin at Ser3 is required for its agonistic action on GHS-R [45]. On the contrary, inhibition of the Ghrelin/GHS-R pathway can reduce food intake, body weight, and adiposity by reducing appetite, increasing energy expenditure, and fat catabolism [46].

An example of an antagonist of GHS-R is liver-expressed antimicrobial peptide-2 (LEAP-2). LEAP-2 is an endogenous non-competitive allosteric antagonist of GHS-R1a [47]. LEAP2 as an endogenous antagonist of GHS-R can inhibit the GHS-R activation by ghrelin and block the ghrelin's effects, like stimulus in food intake, GH release, and maintenance of viable glucose levels during chronic caloric restriction [48]. LEAP-2 is recognized as an endogenous blocker of GHS-R1a. The activity of GHS-R1a is regulated by two counter-regulatory endogenous ligands, namely ghrelin (activation) and LEAP-2 (inhibition) [49]. LEAP-2 acts either as a competitive ghrelin antagonist or an inverse agonist of constitutive GHS-R1a activity. LEAP-2 can block ghrelin's effects on the stimulus of food intake and hormonal secretion. In circulation, LEAP-2 displays an inverse activity to ghrelin, then increases the stimulus of food intake and obesity (positive energy balance) and decreases upon fasting and weight loss (negative energy balance). Thus, the LEAP-2/ghrelin molar ratio varies depending on the energy status, and modulation of this ratio conversely influences energy intake [49].

3.3. *Molecular interactions between xanthorrhizol either with GOAT or GHS-R*

### 3.3.1. *Ligand preparation*

The canonical SMILES data of xanthorrhizol was obtained from PubChem, and then the 3D structure of XNT was created using Marvin Sketch software. Native ligands attached to the Ghrelin and GOAT models were separated using Discovery Studio 2021 software. The ligands used in this study were optimized using MOE 2019.0102 software.

### 3.3.2. *Molecular interaction between ghrelin and desacyl ghrelin (DAG)*

Molecular docking between ghrelin and octanoic acid; ghrelin and xanthorrhizol, GOAT protein; and XNT were performed using the PatchDock server. The clustering RMSD parameter was set as 4 Å, and the complex type was set as "protein-small ligand" for protein-small ligand docking and "default" for protein-protein docking. The best results for protein-protein docking [50] were refined using the FIREDock server for further analysis. A higher docking score may indicate less steric hindrance, and an ACE score may suggest a spontaneous reaction between protein and ligand [51].

Ghrelin protein sequences with access codes NP\_001289751 were obtained by searching the NCBI database using RefSeq and Homo sapiens filter. Protein modeling of the sequence data in fasta format using the SWISS-MODEL server (<https://swissmodel.expasy.org/>) [52], and the modeling results from the SWISS-MODEL server is a ghrelin protein model with 100% similarity [53] to the protein bank database with access code 7f9y (<https://www.rcsb.org/structure/7F9Y>).

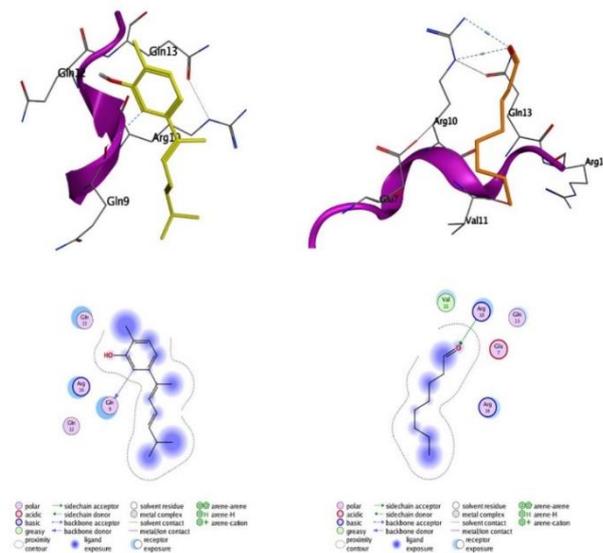
Docking analysis revealed that the molecular ligand interaction of XNT with DAG is not in the same site as octanoic acid (Fig. 4, Table 1). Octanoic acid attaches to Ser 2, but XNT attaches to Gln 9.

### 3.3.3. *Molecular interaction between xanthorrhizol and GOAT*

GOAT (ghrelin O-acyltransferase) protein sequences with access codes NP\_001094386.1 were obtained by searching the NCBI database using RefSeq and Homo sapiens filter. Protein modeling of the sequence data in fasta format using the SWISS-MODEL server (<https://swissmodel.expasy.org/>), and the modeling results from the SWISS-MODEL server is a GOAT protein model with 100% similarity to the protein

**Table 1.** Molecular docking prediction of Ghrelin with Octanoic Acid and Xanthorrhizol

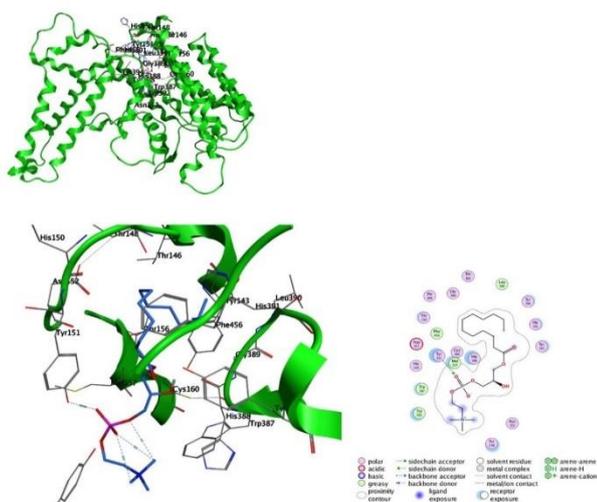
No	Compound	Docking Score	Ligand Interaction	Type interaction	Distance
1	Octanoic Acid	1982	Arg 10	H-acceptor	3.23
			Arg 10	H-acceptor	3.04
2	Xanthorrhizol	3002	Gln 9	H-donor	3.11



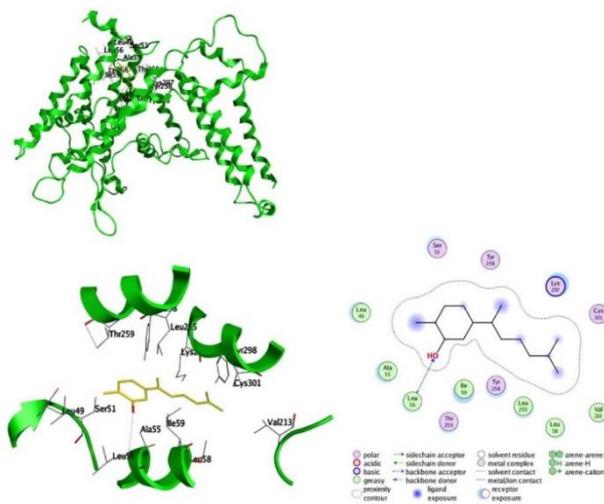
**Figure 4.** Interaction of Ghrelin with Xanthorrhizol (left) and Ghrelin with Octanoic Acid (right)

bank database with access code 7f3x (<https://www.rcsb.org/structure/7F3X>).

The molecular interaction of XNT with GOAT (Fig. 6 and 7) is compared with the interaction of L-alpha-lysophosphatidylcholine (LAP) (Fig. 5). XNT can interact with Thr 143 of GOAT. This site does not have interaction with LAP (Table 2).



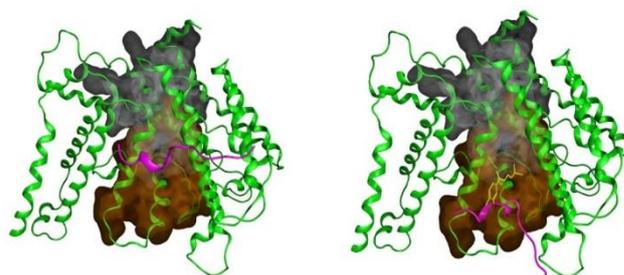
**Figure 5.** Ligand interaction of GOAT with LAP (above, left), GOAT with LAP siteview (above, right), GOAT dengan LAP ligand interaction (below, right)



**3.3.4. Molecular interaction between xanthorrhizol and GHS-R**

Xanthorrhizol is supposed to be a responsible enzyme for satiety modulation. However, it is not yet clear whether XNT is an agonist, antagonist, or inverse

**Figure 6.** Ligand interaction of GOAT with Xanthorrhizol (above, left), GOAT with Xanthorrhizol site view (below, left), and GOAT with Xanthorrhizol ligand interaction (below, right)



**Figure 7.** GOAT interaction with ghrelin; without Xanthorrhizol (left); with Xanthorrhizol (right). The gray and orange surface represent the two largest pocket in GOAT.

agonist of GHS-R. Octanoic acid binds with Gln120 and Arg 102 (Fig. 8, 9 and 10). XNT has binding with residues Asn 305 and Phe 312 (Fig. 11 and Table 3).

**4. Conclusions**

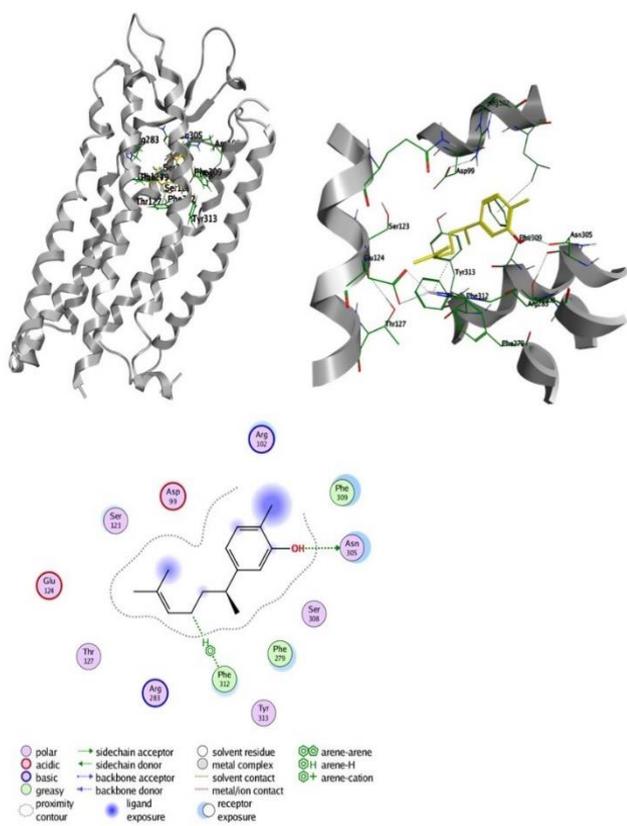
This study shows that xanthorrhizol can target the ghrelin system (DAG, GOAT, and GHS-R). However,

**Table 2.** GOAT Docking

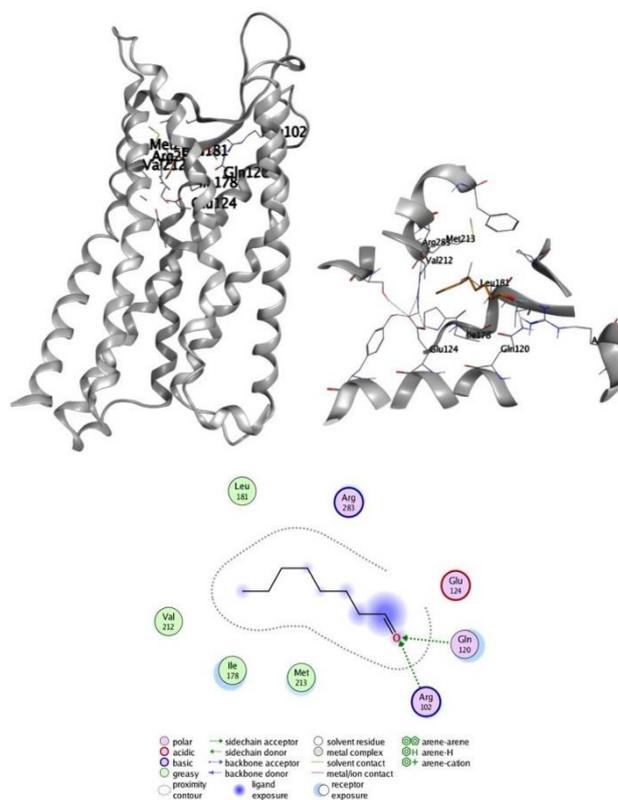
No	Compound	Docking Score	ACE	Ligand Interaction	Type interaction	Distance
1	LAP	6272	-166.21	Tyr 151	H-donor	3.25
				Cys 160	H-acceptor	3.29
				His 388	H-donor	3.47
				His 388	H-donor	3.56
2	Xanthorrhizol	4576	-167.95	Leu 56	H-acceptor	3.81
3	Ghrelin	10220	-183.08			
4	Ghrelin – Xanthorrhizol	– 9932	-294.25			

**Table 3.** GHRS Docking

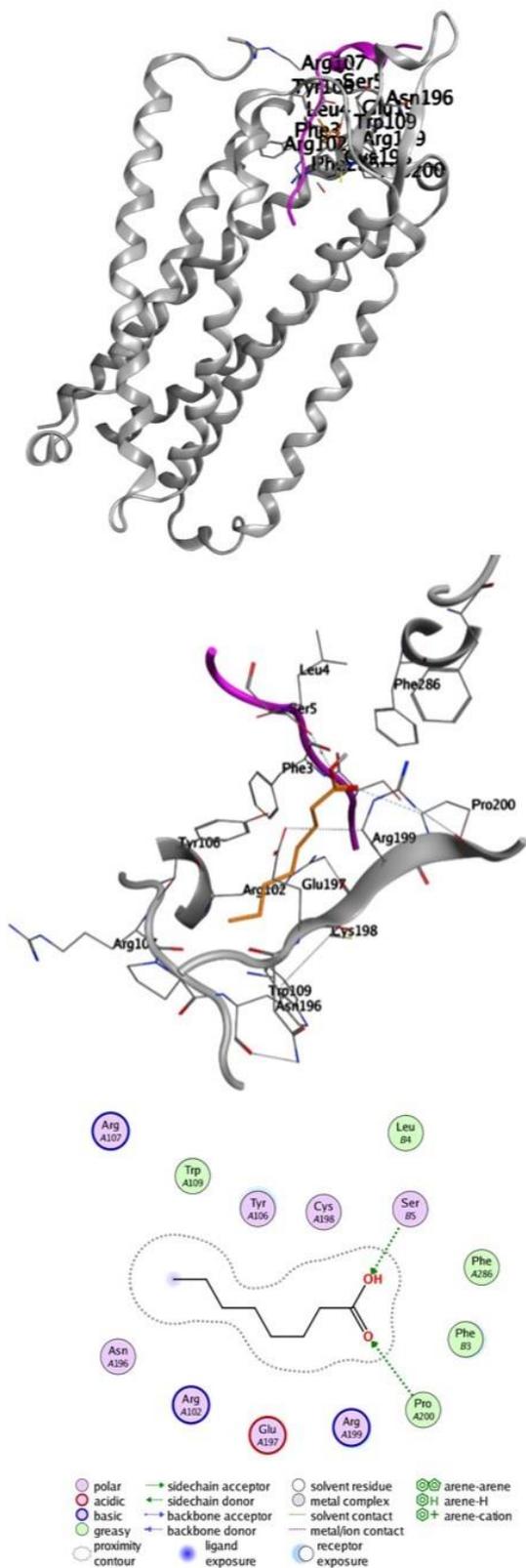
No	Compound	$\Delta G$ ( $\frac{kJ}{mol}$ )	rmsd	Ligand Interaction	Type interaction	Distance	ACE
1	Octanoic Acid	-4.6131	1.2333	Arg 102	H-acceptor	2.79	
				Arg 102	H-acceptor	3.31	
				Gln 120	H-acceptor	3.07	
2	Xanthorrhizol	-6.1115	1.6579	Asn 305	H-donor	3.02	
				Phe 312	H-pi	4.04	
3	Ghrelin – Octanoic Acid	-2.2279	1.2463	Pro 200 (GHRS)	H-acceptor	3.54	-4.45
				Ser 5 (Ghrelin)	H-acceptor	2.89	
4	Ghrelin – Xanthorrhizol	-5.3238	2.2313	Arg 10	H-acceptor	3.11	-4.01



**Figure 8.** Ligand interaction of GHRS with Xanthorrhizol (above, left), GHRS with Xanthorrhizol siteview (above, right), GHRS with Xanthorrhizol (below)

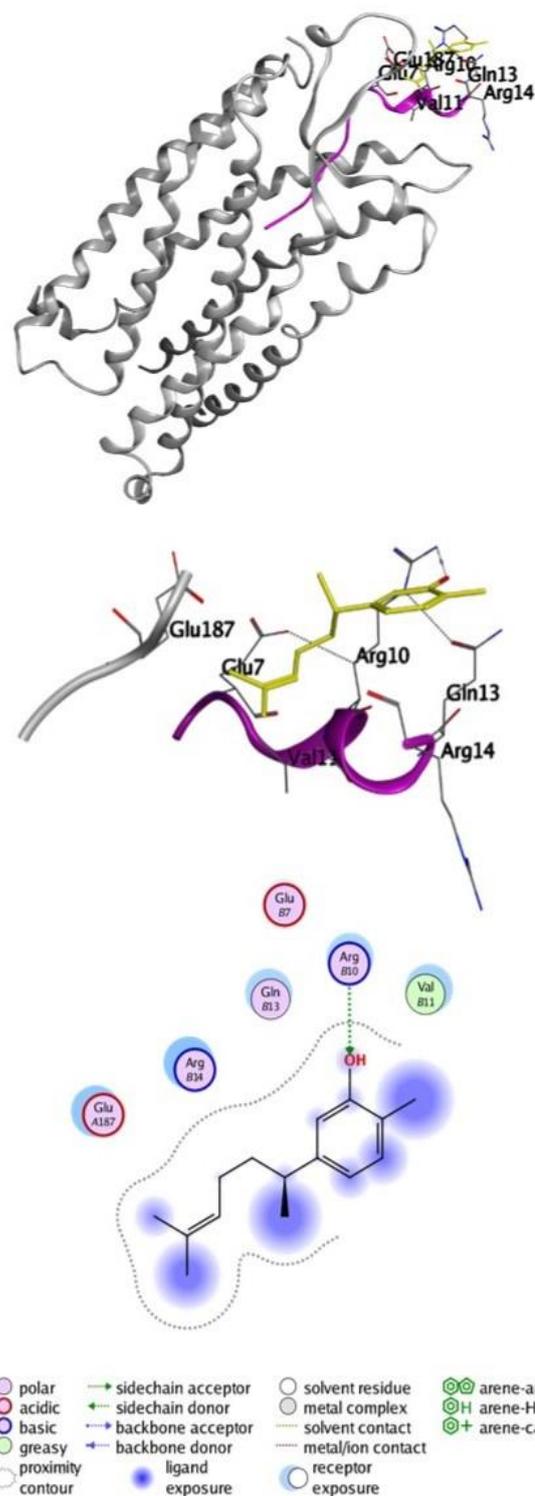


**Figure 9.** Ligand Interaction of GHRS with OAC (above, left), GHRS with OAC siteview (above, right), GHRS with OAC (below)



**Figure 10.** Ligand interaction of GHSR with Ghrelin and Octanoic acid (above, left), GHSR with Ghrelin and Octanoic acid siteview (above, right), GHSR with Ghrelin and Octanoic acid (below, left).

XNT cannot modulate satiety via G-GOAT-GHS-R system even if it can bind with Arg10 of desacyl



**Figure 11.** Ligand interaction of GHSR with Ghrelin and Xanthorrhizol (above, left), GHSR with Ghrelin and Xanthorrhizol-siteview (above, right), GHSR with Ghrelin and Xanthorrhizol (below, left).

ghrelin (DAG peptide). This binding site is far from the binding site (Ser2) of octanoic acid. Therefore, XNT does not inhibit the binding of DAG with octanoic acid.

XNT interacts with GOAT in the active site of octanoic-CoA. This interaction means that XNT is a competitive inhibitor of GOAT activity. It has octanoyl-CoA competitive inhibitory activity and decreases acyl ghrelin production. XNT can interact with GHS-R in the small cavity II where small metabolite agonists enter. This interaction is then interfering with the signaling active GHS-R. Therefore, XNT can be considered an agonist or antagonist of GHS-R.

### List of Abbreviations

AG	: Acyl ghrelin (AG) or ghrelin
AMPK	: AMP-activated protein kinase
BBB	: Blood brain barrier
DAG	: Des-acyl Ghrelin
UAG	: Unacylated or des-acyl Ghrelin (DAG)
G3S	: Ghrelin/GOAT/GHS-R system
GH	: Growth hormone
GI	: Gastrointestinal
GOAT	: Ghrelin-O-acyl transferase
GHRP-6	: GH-releasing hormone
GHS-R	: Growth hormone secretagogue receptor
GPCRs	: G-protein-coupled receptors
LAP	: L-Alpha-Lysophosphatidylcholine
MBOAT	: Membrane-bound O-acyltransferase
NSAIDs	: Non-steroid anti-inflammatory drugs
XNT	: Xanthorrhizol

### Author Contributions

Docking analysis, A.L.; checked and confirmed the xanthorrhizol-related issue, A.S.; overall coordination and editing the final manuscript, KHT.

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### Conflicts of interest

all authors have no potential conflict of interest.

### References

- Oon, S.F.; Nallappan, M.; Kassim, N.K.; Shohaimi, S.; Sa'ariwijaya, M.S.; Tee, T.T.; Cheah Y.H. Hypolipidemic activities of xanthorrhizol purified from centrifugal TLC. *Biochem. Biophys. Res. Commun.* 2016, 478(3), 1403-8. DOI: 10.1016/j.bbrc.2016.08.136.
- Rahmat, E.; Lee, J.; Kang, Y.; Javanese turmeric (*Curcuma xanthorrhiza* Roxb.): ethnobotany, phytochemistry, biotechnology, and pharmacological activities. *Evidence-based Complement. Alt. Med.* 2021, 99608139960813. DOI: 10.1155/2021/9960813.
- Ismail, N.; Pihie, A.H.; Nallapan, M. Xanthorrhizol induces apoptosis via the up-regulation of bax and p53 in HeLa cells. *Anticancer Res*, 2005, 25(3b), 2221-7.
- Oon, S.F.; Nallappan, M.; Kassim, N.K.; Shohaimi, S. Sa'ariwijaya, M.S.; Tee, T.T.; Cheah Y.H. Xanthorrhizol: a review of its pharmacological activities and anticancer properties. *Cancer Cell Int.* 2015, 15, 100. DOI: 10.1186/s12935-015-0255-4.
- Davis, J.F.; Perello, M.; Choi, D.L.; Magrisso, I.J.; Kirchner, H.; Pfluger, P.T.; Tschoep, M. Zigman, J.M.; Benoit, S.C. GOAT induced ghrelin acylation regulates hedonic feeding. *Horm. Behav.* 2012, 62(5), 598-604. DOI: 10.1016/j.yhbeh.2012.08.009.
- Inui, A. Ghrelin: An orexigenic and somatotrophic signal from the stomach. *Nature Rev. Neurosci.*, 2001, 2(8), 551-560. DOI: 10.1038/35086018.
- Davis, T.R., Pierce, M.R.; Novak, S.X.; Houglan, J.L. Ghrelin octanoylation by ghrelin oacyltransferase: protein acylation impacting metabolic and neuroendocrine signalling. *Open Biol.* 2021, 11(7), 210080. DOI: 10.1098/rsob.210080.
- Sato, T., Ida, Y.; Nakamura, Y.; Shiimura, K.; Kangawa, M.; Kojima. Physiological roles of ghrelin on obesity. *Obes. Res. Clin. Pract.* 2014, 8(5), e405-13. DOI: 10.1016/j.orcp.2013.10.002.
- Sato, T., Nakamura, Y.; Shiimura, Y.; Ohgusu, H.; Kangawa, K.; Kojima, M. Structure, regulation and function of ghrelin. *J. Biochem.* 2012, 151(2), 119-28. DOI: /10.1093/jb/mvr134.
- Darling, J.E.; Prybolsky, E.P.; Sieburg, M.; Houglan, J.L. A fluorescent peptide substrate facilitates investigation of ghrelin recognition and acylation by ghrelin o-acyltransferase. *Anal. Biochem.* 2013, 437(1), 68-76. DOI: 10.1016/j.ab.2013.02.013.
- Asakawa, A.; Ataka, K.; Fujino, K.; Chen, C.Y.; Kato, I.; Fujimiya, M.; Inui, A. Ghrelin family of peptides and gut motility. *J. Gastroen. Hepatol.*, 2011. 26 (Suppl 3), 73-4. DOI: 10.1111/j.1440-1746.2011.06638.x.
- Date, Y. Ghrelin and the vagus nerve. *Methods Enzymol.* 2012, 514, 261-9. DOI: 10.1016/b9780-12-381272-8.00016-7.

13. Rhea, E.M.; Salameh, T.S.; Gray, S.; Niu, J.; Banks, W.A.; Tong, J., Ghrelin transport across the bloodbrain barrier can occur independently of the growth hormone secretagogue receptor. *Mol. Metab.* 2018, 18, 88-96. DOI: 10.1016/j.molmet.2018.09.007.
14. Delhanty, P.J., Neggers, S.J.; van der Lely, A.J. Should we consider des-acyl ghrelin as a separate hormone and if so, what does it do? *Front. Horm. Res.* 2014, 42, 163-74. DOI: 10.1159/000358345.
15. Pinkney, J.; The role of ghrelin in metabolic regulation. *Curr. Opin. Clin. Nutr. Metab. Care.* 2014, 17(6), 497-502. DOI: 10.1097/mco.000000000000101.
16. Fujimiya, M.; Asakawa, A., Ataka, K.; Chen, C.Y.; Kato, I.; Inui, A. Ghrelin, des-acyl ghrelin, and obestatin: regulatory roles on the gastrointestinal motility. *Int. J. Pept.* 2010. DOI: 10.1155/2010/305192.
17. Gil-Campos, M.; Aguilera, C.M.; Cañete, R.; Gil, A.; Ghrelin: a hormone regulating food intake and energy homeostasis. *Br. J. Nutr.* 2006, 96(2), 201-26. DOI: 10.1079/bjn20061787.
18. Hosoda, H., M. Kojima, H. Matsuo, and K. Kangawa, Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun*, 2000, 279(3), 909-13. DOI: 10.1006/bbrc.2000.4039.
19. Filigheddu, N.; Gnocchi, V.F.; Coscia, M.; Cappelli, M.; Porporato, P.E.; Taulli, R.; Traini, S.; Baldanzi, G.; Chianale, F.; Cutrupi, S.; Arnoletti, E.; Ghè, C.; Fubini, A.; Surico, N.; Sinigaglia, F.; Ponzetto, C.; Muccioli, G.; Crepaldi, T.; Graziani, A. Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells. *Mol. Biol. Cell.* 2007, 18(3), 986-94. DOI: 10.1091/mbc.e06-05-0402.
20. Zhao, T.J.; Liang, G.; Li, R.L.; Xie, X.; Sleeman, M.W.; Murphy, A.J.; Valenzuela, D.M.; Yancopoulos, G.D.; Goldstein, J.L.; Brown, M.S. Ghrelin o-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc. Natl. Acad. Sci. USA.* 2010, 107(16), 7467-72. DOI: 10.1073/pnas.1002271107.
21. Yang, J.; Zhao, T.J.; Goldstein, J.L.; Brown, M.S. Inhibition of ghrelin o-acyltransferase (GOAT) by octanoylated pentapeptides. *Proc. Natl. Acad. Sci. USA.* 2008, 105(31), 10750-5. DOI: 10.1073/pnas.0805353105.
22. Yoneyama-Hirozane, M.; Deguchi, K.; Hirakawa, T.; Ishii, T.; Odani, T.; Matsui, J.; Nakano, Y.; Imahashi, K.; Takakura, N.; Chisaki, I.; Takekawa, S.; Sakamoto, J. Identification and characterization of a new series of ghrelin o-acyl transferase inhibitors. *SLAS. Discov.* 2018, 23(2), 154163. DOI: 10.1177/2472555217727097.
23. Gahete, M.D.; Córdoba-Chacón, J.; Salvatori, R.; Castaño, J.P.; Kineman, R.D.; Luque, R.M. Metabolic regulation of ghrelin oacyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach. *Mol. Cell. Endocrinol.* 2010, 317(1-2), 154-60. DOI: 10.1016/j.mce.2009.12.023.
24. Isokawa, M.; Ghrelin-o-acyltransferase (GOAT) acylates ghrelin in the hippocampus. *Vitam. Horm.* 2022, 118, 369-392. DOI: 10.1016/bs.vh.2021.11.008.
25. Houglund, J.L.; Ghrelin octanoylation by ghrelin oacyltransferase: unique protein biochemistry underlying metabolic signaling. *Biochem. Soc. Trans.* 2019, 47(1), 169-178. DOI: 10.1042/bst20180436.
26. Ohgusu, H.; Takahashi, T.; Kojima, M.; Enzymatic characterization of GOAT, ghrelin O-acyltransferase. *Methods Enzymol.* 2012, 514, 147-63. DOI: 10.1016/b978-0-12-381272-8.00010-6.
27. Ohgusu, H.; Shirouzu, K.; Nakamura, Y.; Nakashima, Y.; Ida, T.; Sato, T.; Kojima, M. Ghrelin o-acyltransferase (GOAT) has a preference for n-hexanoyl-CoA over n-octanoylCoA as an acyl donor. *Biochem. Biophys. Res. Commun*, 2009, 386(1), 153-8. DOI: 10.1016/j.bbrc.2009.06.001.
28. Gutierrez, J.A.; Willency, J.A.; Knierman, M.D.; Coskun, T.; Solenberg, P.J.; Perkins, D.R.; Higgs, R.E.; Hale, J.E. From ghrelin to ghrelin's o-acyl transferase. *Methods Enzymol.* 2012, 514, 129-46. DOI: 10.1016/b978-0-12-381272-8.00009-x.
29. Khatib, M.N.; Gaidhane, S.; Gaidhane, A.M.; Simkhada, P.; Zahiruddin, Q.S., Ghrelin o acyl transferase (GOAT) as a novel metabolic regulatory enzyme. *J. Clin. Diagn. Res.* 2015, 9(2), 01-5. DOI: 10.7860/jcdr/2015/9787.5514.
30. Barnett, B.P.; Hwang, Y.; Taylor, M.S.; Kirchner, H. Pfluger, P.T. Bernard, V. Lin, Y.Y. Bowers, E.M.; Mukherjee, C.; Song, W.J.; Longo, P.A.; Leahy, D.J.; Hussain, M.A.; Tschöp, M.H.; Boeke, J.D.; Cole, P.A. Glucose and weight control in mice with a designed ghrelin o-acyltransferase inhibitor. *Science*, 2010, 330(6011), 1689-92. DOI: 10.1126/science.1196154.
31. Campaña, M.B., Irudayanathan, F.J.; Davis, T.R.; McGovern-Gooch, K.R.; Loftus, R.; Ashkar, M.; Escoffery, N.; Navarro, M.; Sieburg, M.A.; Nangia, S.; Houglund, J.L. The ghrelin o-acyltransferase structure reveals a catalytic channel for transmembrane hormone acylation. *J. Biol. Chem.* 2019, 294(39), 1416614174. DOI: 10.1074/jbc.AC119.009749.
32. Sieburg, M.A.; Cleverdon, E.R.; Houglund, J.L. Biochemical assays for ghrelin acylation and inhibition of ghrelin o-acyltransferase. *Methods Mol. Biol.* 2019, 227-241. DOI: 10.1007/978-14939-9532-5\_18.
33. Du, G.M.; Luo, B.P.; Hu, Z.H.; Wu, J.G.; Yan, W.M.; Han, Z.Q.; Zhang, Y.H.; Liu, M.J. The effect of ghrelin o-acyltransferase inhibitor on gastric H(+)-K(+)-ATPase activity and GOAT/ghrelin system in gastric mucosal cells in vitro. *Gen. Comp. Endocrinol.* 2018, 267, 167-171. DOI: /10.1016/j.ygcen.2018.06.020.

34. Taylor, M.S.; Hwang, Y.; Hsiao, P.Y.; Boeke, J.D.; Cole, P.A. Ghrelin o-acyltransferase assays and inhibition. *Methods Enzymol.* 2012, 514, 20528. DOI: 10.1016/b978-0-12-381272-8.00013-1.
35. Li, Z.; Mulholland, M.; Zhang, W.; Ghrelin oacyltransferase (GOAT) and energy metabolism. *Sci. China Life Sci.* 2016, 59(3), 281-91. DOI: 10.1007/s11427-015-4973-6.
36. Micioni Di Bonaventura, E.; Botticelli, L.; Del Bello, F.; Giorgioni, G.; Piergentili, A.; Quaglia, W.; Cifani, C.; Micioni Di Bonaventura, M.V. Assessing the role of ghrelin and the enzyme ghrelin o-acyltransferase (GOAT) system in food reward, food motivation, and binge eating behavior. *Pharmacol Res.* 2021, 172, 105847. DOI: /10.1016/j.phrs.2021.105847.
37. Bridges, T.M.; Lindsley, C.W. G-protein-coupled receptors: from classical modes of modulation to allosteric mechanisms. *ACS Chem. Biol.* 2008, 3(9), 530-41. DOI: 10.1021/cb800116f.
38. Yin, Y.; Li, Y.; Zhang, W. The growth hormone secretagogue receptor: its intracellular signaling and regulation. *Int. J. Mol. Sci.* 2014, 15(3), 4837-55. DOI: 10.3390/ijms15034837.
39. Albarrán-Zeckler, R.G.; Smith, R.G. The ghrelin receptors (GHS-R1a and GHS-R1b). *Endocr. Dev.* 2013, 25, 5-15. DOI: 10.1159/000346042.
40. Airapetov, M.I.; Eresko, S.O.; Lebedev, A.A.; Bychkov, E.R.; Shabanov, P.D. Expression of the growth hormone secretagogue receptor 1a (GHS-R1a) in the brain. *Physiol. Rep.* 2021, 9(21), e15113. DOI: 10.14814/phy2.15113.
41. Giorgioni, G.; Del Bello, F.; Quaglia, W.; Botticelli, L.; Cifani, C.; Micioni E. Di Bonaventura, M.V.; Di Bonaventura, M.; Piergentili, A. Advances in the development of nonpeptide small molecules targeting ghrelin receptor. *J. Med. Chem.* 2022, 65(4), 3098-3118. DOI: 10.1021/acs.jmedchem.1c02191.
42. Moulin, A.; Ryan, J.; Martinez, J.; Fehrentz, J.A.; Recent developments in ghrelin receptor ligands. *Chem. Med. Chem.* 2007, 2(9), 1242-59. DOI: 10.1002/cmdc.200700015.
43. Bennett, K.A.; Langmead, C.J.; Wise, A.; Milligan, G. Growth hormone secretagogues and growth hormone releasing peptides act as orthosteric super-agonists but not allosteric regulators for activation of the G protein Galpha(o1) by the Ghrelin receptor. *Mol. Pharmacol.* 2009, 76(4), 802-11. DOI: 10.1124/mol.109.056101.
44. Holst, B.; Brandt, E.; Bach, A.; Heding, A.; Schwartz, T.W. Nonpeptide and peptide growth hormone secretagogues act both as ghrelin receptor agonist and as positive or negative allosteric modulators of ghrelin signaling. *Mol. Endocrinol.* 2005, 19(9), 2400-11. DOI: /10.1210/me.2005-0059.
45. Liu, H.; Sun, D.; Myasnikov, A.; Damian, M.; Baneres, J.L.; Sun, J.; Zhang, C. Structural basis of human ghrelin receptor signaling by ghrelin and the synthetic agonist ibutamoren. *Nat. Commun.* 2021, 12(1), 6410. DOI: 10.1038/s41467-021-26735-5.
46. Soares, J.B.; Roncon-Albuquerque, R.; Leite Moreira, A. Ghrelin and ghrelin receptor inhibitors: agents in the treatment of obesity. *Expert Opin. Ther. Targets.* 2008, 12(9), 1177-89. DOI: 10.1517/14728222.12.9.1177.
47. Barja-Fernández, S.; Lugilde, J.; Castelao, C.; Vázquez-Cobela, R.; Seoane, L.M.; Diéguez, C.; Leis, R.; Tovar, S.; Circulating LEAP-2 is associated with puberty in girls. *Int. J. Obes (Lond).* 2021, 45(3), 502-514. DOI: 10.1038/s41366-020-00703-3.
48. Ge, X.; Yang, H.; Bednarek, M.A.; Galon-Tilleman, H.; Chen, P.; Chen, M.; Lichtman, J.S.; Wang, Y.; Dalmás, O.; Yin, Y.; Tian, H.; Jermutus, L.; Grimsby, J.; Rondinone, C.M.; Konkar, A.; Kaplan, D.D. LEAP2 Is an Endogenous Antagonist of the Ghrelin Receptor. *Cell Metab.* 2018, 27(2), 461469.e6. DOI: 10.1016/j.cmet.2017.10.016.
49. Lu, X.; Huang, L.; Huang, Z.; Feng, D.; Clark, R.J.; Chen, C. LEAP-2: An emerging endogenous ghrelin receptor antagonist in the pathophysiology of obesity. *Front. Endocrinol (Lausanne).* 2021, 12, 717544. DOI: 10.3389/fendo.2021.717544.
50. Schneidman-Duhovny, D.; Inbar, Y.; Nussinov, R.; Wolfson, H.J.; PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res.* 2005, 33(Web Server issue): W363-7. DOI: /10.1093/nar/gki481.
51. Zarei, M.; Abidin, N.B.Z.; Auwal, S.M.; Chay, S.Y.; Haiyee, Z.A.; Sikin, A.M.; Saari, N. Angiotensin converting enzyme (ACE)-peptide interactions: inhibition kinetics, in silico molecular docking and stability study of three novel peptides generated from palm kernel cake proteins. *Biomolecules.* 2019, 9(10), DOI: 10.3390/biom9100569.
52. Waterhouse, A.; Bertoni, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T. de Beer, T.A.P.; Rempfer, C.; Bordoli, L.; Lepore, R.; Schwede, T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018, 46(W1, W296-w303). DOI: 0.1093/nar/gky427.
53. Benkert, P.; Biasini, M.; Schwede, T. Toward the estimation of the absolute quality of individual protein structure models. *Bioinform.* 2011, 27(3), 343-50. DOI: 10.1093/bioinformatics/btq662.