ORIGINAL ARTICLE

In silico identification of natural compounds from virgin coconut oil as potential ligand peroxisome proliferator-activated receptor-gamma as preventive food leads against colitis: Is it really work?

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ABSTRACT

Ulcerative colitis (UC) is an inflammation of the large intestine characterized by diarrhea with blood. UC has a more extensive manifestation in children. Current therapy has not given satisfactory results. This is the basis for the need for preventive therapy to reduce the morbidity and mortality of UC in children. Virgin coconut oil (VCO) is a viable dietary supplement option due to its ability to act as a peroxisome proliferator-activated receptor (PPAR) ligand, inhibiting the release of pro-inflammatory cytokines. The aim of this study was to determine natural compounds from VCO that have the potential to prevent colitis using a docking-based virtual screening approach. Quantitative structure-activity relationship analysis was used to find out how similar the input compounds and the database were. Docking is done using AutoDockTools 1.5.6. The algorithm used is the Lamarckian Genetic Algorithm (4.2). PPAR-gamma (PPAR-γ) was used as the target protein in a complex with rosiglitazone (ID PDB: 7AWC). PyMol 2.5.1 was used to prepare and visualize three-dimensional data, and the amino acid interactions were visualized using Discovery Studio 2021 Clients. It was found that linoleic acid and oleic acid in VCO have anti-inflammatory effects with predictive values of 0.73 and 0.614, respectively, and that they stop tumor necrosis factor (TNF) expression with predictive values of 0.751 and 0.724. The result of molecular docking showed that the VCO compound was able to interact with the same residue as the control. VCO reduces inflammation by acting as a PPAR-γ and TNF expression inhibitor.

Key words: Anti-inflammatory, *in silico*, ulcerative colitis, virgin coconut oil

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INTRODUCTION

Ulcerative colitis (UC) is a type of inflammatory bowel disease that causes the mucosa of the colon to become inflamed. It can cause a wide range of clinical symptoms.[1] UC can affect the rectum, part or all of the large intestine, can

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affect any age, and is most common in the second and third decades of life.[2] About 20%–30% of cases are diagnosed in childhood or adolescence.[3]

The incidence and prevalence of UC differ in each region. UC is found in developed countries and is thought to be related to intestinal infections, diet, and lifestyle there.[4] In Europe and North America, the prevalence of UC ranges from 7.9 to 20.6 per 100,000 people. In Asia and other developing countries, it is 15.0 per 100,000 people.[5] UC becomes a global problem when there is an increase in its incidence at a young age. Most people get it when they are teenagers, and a quarter of cases are found before the age of 18.[6,7]

The symptoms of UC, as well as the results of endoscopy, radiology, and histopathology, are used to make a diagnosis.[8] Symptoms of UC in children can be abdominal pain, diarrhea, and systemic symptoms in the form of weight loss, delayed growth, and depression.[3] Children with UC have a wider range of symptoms and a more aggressive disease course than adults. Compared to the general population, they also have twice the risk of cancer.[9,10] No one knows for sure what causes UC, but there is evidence that genetic, environmental, infectious, immune, and interacting factors all play a role in the disease's development and worsening.[11] UC is most likely caused by a mix of genetic and environmental factors that cause the intestinal barrier epithelium to break down and the immune response to the gut microbiota to be out of whack.[12]

In patients with UC, contemporary therapies like corticosteroids have not demonstrated a beneficial response and can result in substantial adverse effects. There is a demand for plant-based alternative medicines with few adverse effects, one of them is virgin coconut oil (VCO). VCO is a processed product made from fresh coconut oil which is extracted by controlled heating or without heating at all and processed without chemicals. VCO has nutritional and therapeutic benefits as an antioxidant, anti-inflammatory, antipyretic, and analgesic. VCO has bioactive components including tocopherols, tocotrienols, and polyphenols, which may be responsible for the above effects.[13] Polyunsaturated fatty acid (PUFA) VCO in the form of oleic acid, linoleic acid, and flavonoids functions as an anti-inflammatory. Linoleic acid itself has attracted the attention of the food and supplement industry because it is an activator of peroxisome proliferator-activated receptor-gamma (PPAR-γ) which is widely used as a diabetes therapy and has potential as an anti-inflammatory.[14]

According to studies on mice given a diet VCO, Lauric acid in VCO functions as a ligand for PPAR-α.[15] Other studies have also mentioned that VCO inhibits PPAR-β/δ in muscle and activates PPAR-a in the liver.[16] Hence, it is likely that VCO also influences colonic PPAR-γ, maintains the balance of intestinal immunity, and provides anti-inflammatory effects. The fact that PUFA contributes to the PPAR signaling pathway and makes VCO an anti-inflammatory makes it a candidate for preventing UC. This study aims to analyze the ability of compounds in VCO to prevent inflammation of the colon using an *in silico* approach. The target protein used was PPAR-γ as a result of X-ray diffraction with ID (PDB: 7AWC), namely PPAR-γ in complex with rosiglitazone. This research can also be used as a starting point for more studies on how to treat UC.

SUBJECTS AND METHODS

Collection of virgin coconut oil content samples

Bioactive compounds from VCO were obtained from journal studies.[13,17,18] After finding the VCO content, information is taken from the PubChem database (pubchem.ncbi.nlm.nih.gov) in the form of an ID code and a simplified molecular-input line-entry system as a sample analysis.[19]

Quantitative structure-activity relationship analysis of active compounds with way2drug

Way2Drug/PASS online (www.way2drug.com/PASSOnline) was used to do a quantitative structure-activity relationship (QSAR) analysis to find out how similar the input compounds and the database were. The score shown by the webserver varies from 0 to 1, which indicates the similarity between the structure of the input compound and the compounds that have been studied to have inflammatory activity.[20] The potential parameters analyzed were related to inflammation.

Target protein analysis

The target protein that can interact with linoleic acid and oleic acid is predicted using the similarity ensemble approach (SEA) target (https://sea.bkslab.org/). The SEA target also uses a similarity approach to predict interactions with target proteins by calculating the Tanimoto coefficient. The minimum cutoff used is 0.57, as suggested in the journal.[21]

Molecular docking

Docking is done using AutoDock in AutoDockTools 1.5.6. The parameter used is the Lamarckian Genetic Algorithm (4.2). The target protein used was PPAR-γ as a result of X-ray diffraction with ID (PDB: 7AWC), namely the crystal structure of PPAR-γ in complex with rosiglitazone. Results of experiments were used for grid box and control. Because there was no experimental protein structure with 5-ASA, 5-ASA was still used as a comparison with rosiglitazone, linoleic acid (PubChem ID: 5280450), oleic acid (PubChem ID: 445639), and 5-ASA (PubChem ID: 4075). Docking is done on the grid box as follows:

Even number of user-specified grid points = 40 x-points 40 y-points 40 z-points

Coordinates of central grid point of maps = (41.391, 3.724, 82.402)

The three-dimensional data were prepared and visualized using PyMol 2.5.1, and the interactions between amino acids were displayed using Discovery Studio 2021 Clients.[22,23]

RESULTS AND DISCUSSION

Collection of virgin coconut oil content samples

Obtained references to compounds contained in VCO in supporting journals which were then analyzed.

Quantitative structure-activity relationship analysis of active compounds with Way2Drug

QSAR is an analysis that uses the principle of structural similarity.[24] The compounds found in the VCO are then inputted into the database and compared with the compounds curated by the Way2Drug Pass Server, which have a certain potential. The more similar the structure, the higher the predicted score. If the predicted value is more than 0.7, then the similarity of the compound is high and the confidence value is also high; more than 0.5 but <0.7, then moderate; and if <0.3, then low.[20] Figure 1 illustrates the analysis's findings.

Based on the QSAR analysis, the compound in VCO is predicted to have high potential as an anti-inflammatory (0.555). This role is also supported by the potential of VCO as a tumor necrosis factor (TNF) expression inhibitor (0.622). TNF is one of the pro-inflammatory cytokines that can trigger inflammation.[25] TNF plays a crucial role in the regulation of innate immune responses. By inducing a signal cascade, TNF can activate several molecular mechanisms associated with the pathogenesis of chronic intestinal inflammation. TNF triggers the secretion of inflammatory mediators and controls the recognition and treatment of invading bacteria. TNF is also involved in the growth, differentiation, and survival of macrophages, as well as in the activation and function of innate immune cells.[26] Linoleic acid and oleic acid have anti-inflammatory roles with predictive values of 0.73 and 0.614, and their roles in TNF expression inhibitors are 0.751 and 0.724, respectively.

A study discovered that TNF-α can inhibit the expression of the PPAR-γ via the nuclear factor-kappa-light-chain-enhancer of activated B-cells (NF-κB) pathway. TNF-α activates both the inhibitor of kappa B kinase (IkKb)/NF-κB and the mitogen-activated protein kinase pathways. It has been demonstrated that the addition of 15dPGJ2 (inhibitor IKK2/IKKb) has the potential to activate PPAR. Furthermore, ssIkBa (IKKb inhibitor) can inhibit NF-κB activation as well as TNF-α's ability to inhibit PPAR-γ. PPAR-γ activity was analyzed using a luciferase reporter on 3T3-L1 cells. The presence of TNF-α can reduce reporter response by 40%.

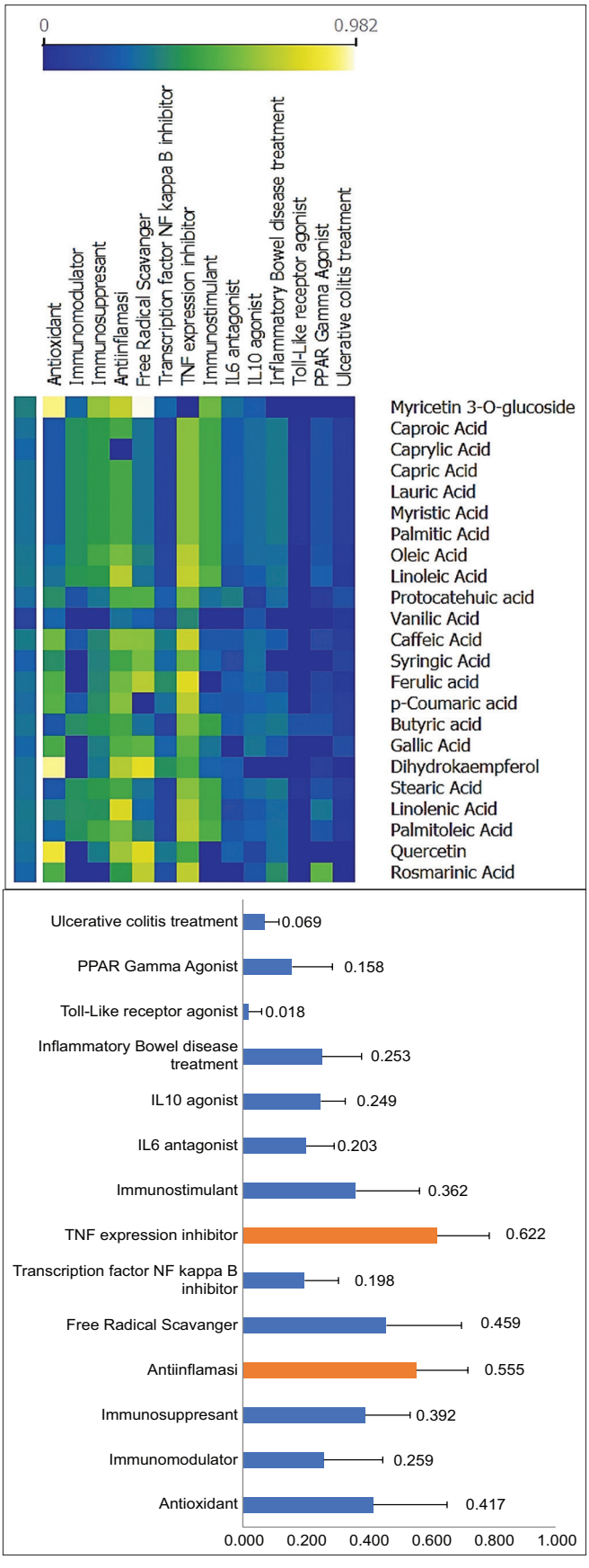

Figure 1: Potential of VCO as treatment of IBD. VCO: Virgin coconut oil, IBD: Inflammatory bowel disease

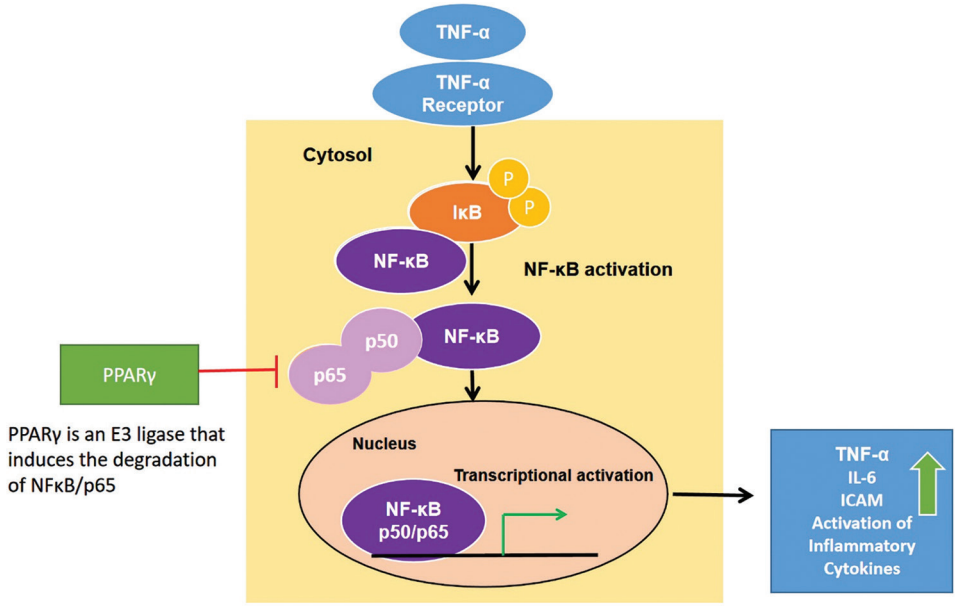

Figure 2: Interactions between PPAR-γ, NF-κB, and TNF-α. PPAR-γ: Peroxisome proliferator-activated receptor-gamma, NF-κB: Nuclear factor-kappa B, TNF-α: Tumor necrosis factor-alpha

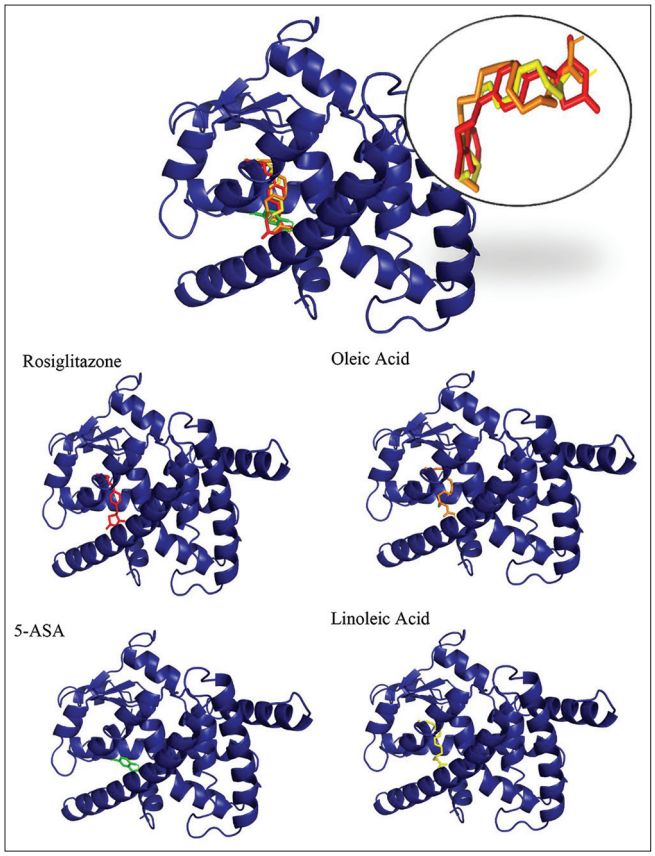

Figure 3: Visualization of ligand-PPAR-ɣ docking results using PyMol. PPAR-ɣ: Peroxisome proliferator-activated receptor-ɣ

This reporter's response describes PPAR-γ activity. Based on this study, it seems likely that NF-κB is the link between TNF-α and PPAR-γ.[27]

NF-κB is a family of dimeric transcription factors consisting of 5 proteins, namely p65 or RelA, RelB, c-Rel, p50, and p52.[28] p65/RelA is regulated by ubiquitin- and proteasome-dependent signaling degradation that terminates NF-κB activation. PPAR-γ can interact with p65, which promotes nuclear export in bacterial responses while inhibiting NF-κB transcriptional activity. A study also discovered that PPAR-γ is an E3 ubiquitin ligase that targets NF-κB/65 to inhibit NF-κB activation by inducing proteasome-dependent degradation of p65.[29]

Target protein analysis

Based on the similarity analysis using SEA target, it shows that linoleic acid and oleic acid found in VCO have a Tanimoto coefficient score to target PPAR-γ of 1, where this score is the maximum score,[30] so that PPAR-γ can be used as a protein target for docking interactions.

This is also supported by several studies, where rosiglitazone, an agonist of PPAR-γ, was reported to cause polarization of M2 macrophages, which contributed to its therapeutic effect on UC.[31] Another study showed that PPAR-γ regulates arginase 1 and interleukin-10 and is involved in M2 macrophage polarization.[32] Studies in animal models and UC patients reveal a role for PPAR in the regulation of inflammation and immune responses, especially in colon epithelial cells.[33] Based on these results, the PPAR pathway can be used to prevent UC by focusing on the M1/M2 macrophage ratio.

Molecular docking

Molecular docking is one of the analyses in bioinformatics to predict the value of binding affinity between two

molecules. The more negative the binding affinity value, the more favorable the bond that occurs.[23] Based on the docking results, linoleic acid is the closest ligand to the original control [Table 1]. The control inhibitor PPAR-γ, rosiglitazone, from X-ray diffraction was re-docked to ensure that the docking results were similar to the experimental results. The parameter seen is the root mean standard deviation (RMSD) value, to see the comparison of

Table 1: Result of molecular docking

Ligand	Estimated free energy of binding (kcal/mol)	Estimated inhibition constant, Ki (uM)	Color
Control rosiglitazone	−10.42	23.03	Red
Oleic acid	−6.24	26.73	Orange
5-ASA	−5.06	194.81	Green
Linoleic acid	−6.42	19.64	Yellow

5-ASA: 5-aminosalicylic acid

Table 2: Amino acid interaction

	Hydrogen bond	Van der Waals	Hydrophobic bond
Control	Ser289, Tyr473*, Cys285, Ile281	His323, Tyr327, Phe363, Gly284, Ile326, Phe282, Leu453, His449*, Gln286*, Leu465	Met364, Leu353, Val339, Met348, Ile341, Arg288, Leu330
Oleic acid	Ser289, His323, Tyr473	Gln286, Leu469, Leu453, Lys367, Met364, Ile281	Met348, Leu330, Tyr327, Ile326, Phe363, His449, Arg288, Cys285, Leu353, Val339, Ile341
5-ASA	Phe282, Ser289, Tyr327	Phe363, His449, Ile326, Gln286, Leu453, Leu469, Tyr473, His323, Leu465	Cys285
Linoleic acid		His323, Lys367, Ile326, Phe363, Met364, Gly284, Ile281, Leu453, Leu469, Gln286*, Leu465	His449*, Tyr327, Leu330, Cys285, Arg288, Ile341, Met348, Leu353, Val339

*Indicates the same amino acid residue as the control and becomes a binding site. 5-ASA: 5-aminosalicylic acid

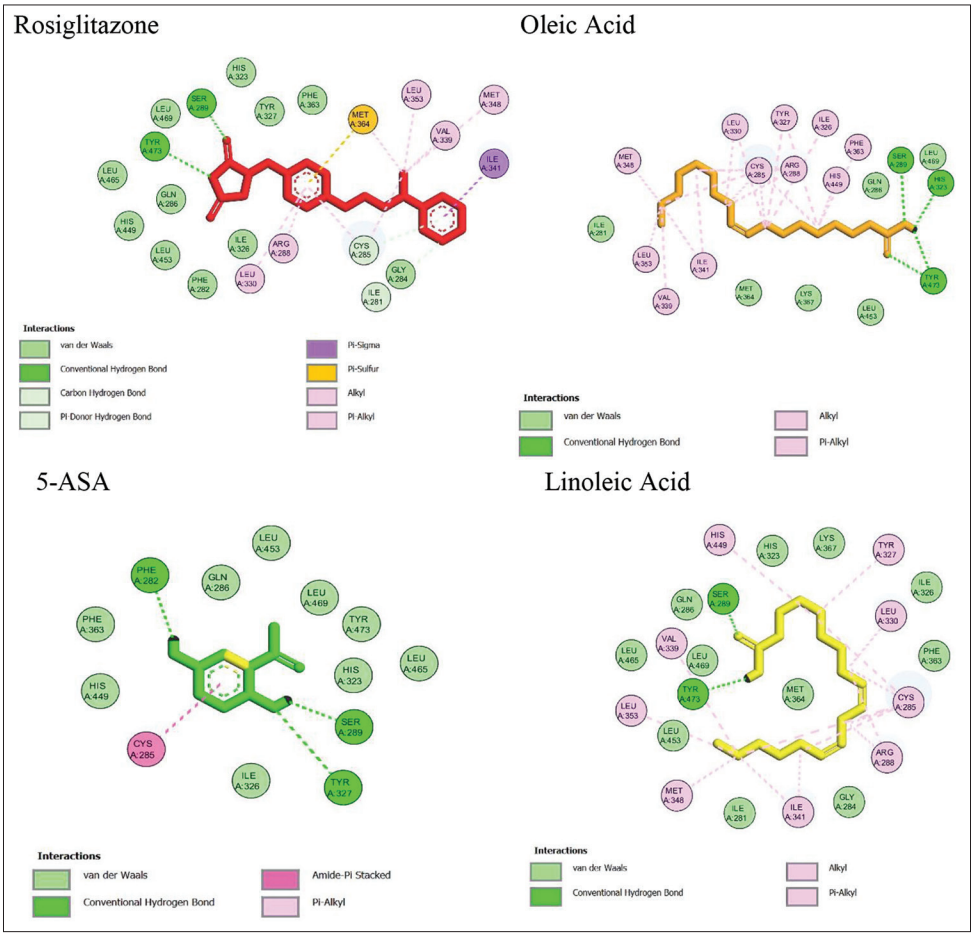

Figure 4: Visualization of amino acid interaction

docking results and experimental results. Docking results are said to be good if the value is below 2 angstroms.[34] The results of the docking control yield a value of 0.945 angstroms, so this result is quite good because the maximum limit of RMSD is 2. The results of linoleic acid are in accordance with the results of QSAR analysis with PASS Server, which is higher than oleic acid, which is shown to be related as a TNF expression inhibitor. TNF ligands can regulate the localization of NF-κB so that it will have an impact on the expression of inflammatory cytokines [Figure 2]. Besides being seen from the RMSD value and binding affinity, the binding pose also affects the docking accuracy [Figure 3].[35] As shown in Table 2 and Figure 4, the VCO compound was able to interact with the same residue as the control. Linoleic acid has a pose and can bind to both the PPAR-γ binding site and control inhibitors.

CONCLUSION

Based on the *in silico* results, VCO can be used as a supplement for the prevention of colitis. VCO, which is composed of linoleic acid and oleic acid, reduces inflammation by acting as a PPAR-ɣ and TNF expression inhibitor.

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Conflicts of interest
There are no conflicts of interest.

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