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# *In silico* molecular docking of quercetin as anti-colorectal cancer agent by inhibiting LT4AH

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**Abstract:** Colorectal cancer is a malignant neoplasm originating from the colon or rectum. Overexpression of leukotriene A4 hydrolase (LTA4H) increases the growth of HCT116 colon cancer cells, therefore, this enzyme becomes an attractive target for commercial drug bestatin. Meanwhile, quercetin is a member of flavonoids possessing a wide variety of anticancer. This study aimed to determine the potential of quercetin as anti-colorectal cancer by inhibiting LTA4H through *in silico* molecular docking. The docking process involved optimizing quercetin structure, preparing LTA4H protein (PDB ID: 3U9W), validating the molecular docking method, and docking quercetin and bestatin on LTA4H. The binding energy of quercetin to LTA4H was -9.57 kcal/mol, while 28P native ligand and bestatin yielded -10.22 kcal/mol and -9.10 kcal/mol, respectively. Based on the binding energy value, quercetin has a potential inhibitory against the LTA4H.

**Keywords:** bestatin, colorectal cancer, LTA4H, molecular docking, quercetin

## Introduction

Colorectal cancers are the third most common type of cancer diagnosed worldwide, accounting for 11% of all cancer diagnoses, according to the GLOBOCAN International Agency for Research on Cancer (IARC) [1]. Colorectal cancer can occur in both men and women. The ratio of men and women with colorectal cancer is 76.7%: 23.3%. The incidence of rectal cancer is more common in men than women, this is due to differences in hormones and also the frequent consumption of alcohol and cigarettes in men [2].

Colorectal cancer is a malignant neoplasm originating from the colon or rectum. Leukotriene A4 hydrolase (LTA4H) has been implicated in colorectal cancer and has been targeted to prevent and treat cancers [3]. Many cases of colorectal cancer are caused by overexpression of LTA4H [4]. LTA4H classically functions as an epoxide hydrolase to produce leukotriene B4 (LTB4) from leukotriene A4 (LTA4). This activity operates in the intracellular compartment and is primarily a function of leukocytes. LTB4 is a highly pro-inflammatory lipid mediator that can exert its activity by binding to BLT1 or BLT2 receptors. LTB4 can promote the recruitment and activation of various cells, including neutrophils, and is thus involved in protection against invading microorganisms and the pathology of various diseases, such as cancer [5].

Chemotherapy, surgery, and radiotherapy are used to treat most colorectal malignancies. Bestatin (ubenimex) is a well-studied LTA4H inhibitor that inhibits the synthesis of LTB4 [6]. In clinical trials, bestatin enhanced the survival of elderly patients with acute myelogeneous leukemia [7]. Recently, bestatin has been investigated in colorectal cancer cells, and results revealed that it suppressed their growth and colony formation [4]. Another option is to explore the potential of effective natural compounds, such as quercetin, as a target for LTA4H in colorectal cancer drug research.

Quercetin is a type of flavonoid compound found in many leafy and fruiting plants. Some studies have investigated the anticancer activity of quercetin [8–10]. In WiDr cells, quercetin has cytotoxic and antiproliferative activity [11]. There has been no research that reported the mechanism of quercetin inhibition on LTA4H. Therefore, this study aimed to determine the potential of quercetin as an anti-colorectal cancer agent against LTA4H through *in silico* molecular docking.

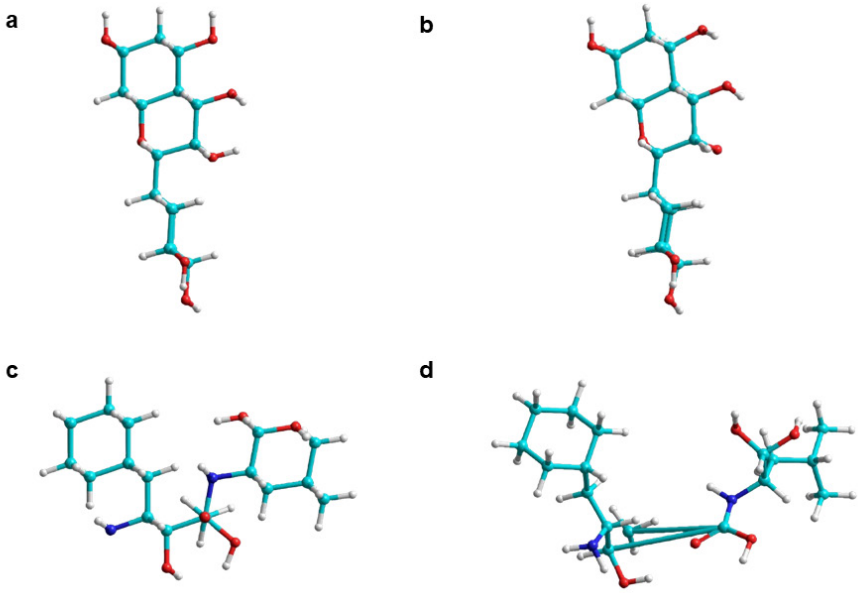


**Figure 1**. The optimized of quercetin and bestatin structure. (a) quercetin single point calculation, (b) quercetin geometry optimization, (c) bestatin single point calculation, (d) bestatin geometry optimization

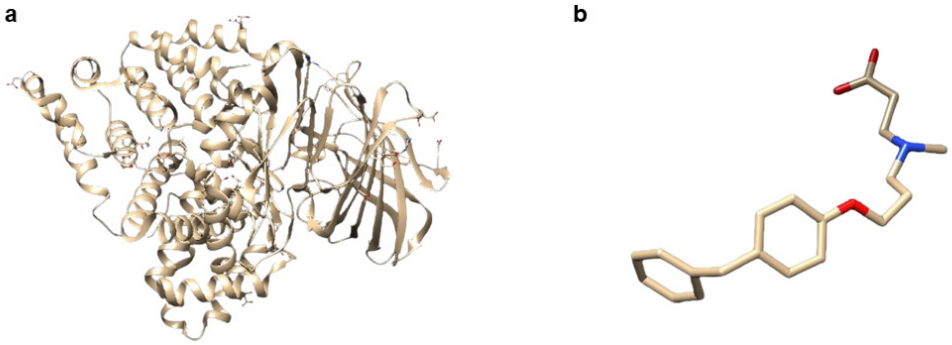


**Figure 2.** Protein LTA4H. (a) chain A of LTA4H protein without native ligand, (b) 28P native ligand

## Methods

### Optimization of quercetin and bestatin structures

The structure of quercetin and bestatin was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) then optimized using the HyperChem 8. Optimization was performed using the AM1 (Austin Model 1) semi-empirical computational method and single point calculations as well as geometry optimization.

### Preparation LTA4H protein

The leukotriene A4 hydrolase (LTA4H) (PDB ID 3U9W) protein was downloaded from https://www.rcsb.org. The LTA4H protein was prepared by separating the protein from 28P (*N*-[3(4-benzylphenoxy)propyl]-*N*-methyl-beta-alanine) native ligand using Chimera 1.10.1 program.

### Molecular docking validation

Validation was carried out using Autodock Tools application (Autodock 4.2 and Autogrid) by redocking 28P native ligand to the prepared LTA4H protein. The grid box size was set and adjusted to x = 40 Å, y = 40 Å, z = 40 Å; grid center x = 29.679 Å, y = 1.546 Å, z = 1.893 Å. The validation was determined by the value of Root Mean Square Deviation (RMSD) value. The RMSD value ≤ 3.0 Å is considered valid [12].

### Docking quercetin and bestatin on LTA4H protein

The optimized structure of quercetin and bestatin was then docked to the target protein using Autodock 4.2 with the same grid box size during validation. The docking results were binding energy and the bonds formed between compounds and target proteins.

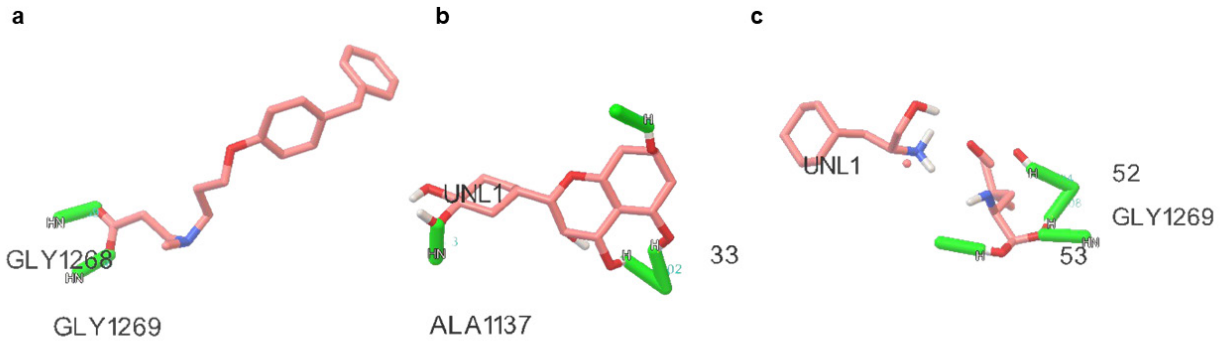


**Figure 3.** Visualization of the interaction. (a) interaction of 28P native ligand to LTA4H, (b) interaction of quercetin on LTA4H, (c) interaction of bestatin to LTA4H

**Table 1.** Docking result of 28P native ligand with LTA4H

| Protein | Ligand | Conformation | RMSD (Å) | Binding Energy (kcal/mol) | Amino acid residue | Groups in hydrogen bonds |
|---|---|---|---|---|---|---|
| LTA4H | 28P | 1 | 2.67 | -10.24 | GLY1268 | HN-OAE |
| | | 2 | 2.16 | -10.27 | GLY1269 | HN-OAE |
| | | 3* | 2.08 | -10.22 | GLY1268 | HN-OAE |
| | | 4 | 2.56 | -10.83 | GLY1269 | HN-OAF |
| | | 5 | 5.12 | -9.06 | GLY1268 | HN-OAE |
| | | 6 | 5.19 | -8.24 | GLY1269 | HN-OAF |
| | | 7 | 4.60 | -9.26 | SER1379 | HN-OAF |
| | | 8 | 2.48 | -10.69 | - | - |
| | | 9 | 2.10 | -10.80 | TRP1315 | HN-OAE |
| | | 10 | 2.53 | -10.69 | GLY1268 | HN-OAF, OAE |

## Results

### Optimization of quercetin and bestatin structures

The optimization of quercetin provided a single point energy calculation of -4676.93 kcal/mol and geometry optimization of -4711.81 kcal/mol. The similar parameters for bestatin were -4743.04 kcal/mol and -5631.02 kcal/mol (Figure 1).

### Preparation LTA4H protein

The LTA4H protein was prepared by separating the protein from the native ligand. LTA4H protein without 28P native ligand and a separate native ligand 28P were visualized in Figure 2.

### Molecular docking validation

The docking validation purposes to determine the conformational similarity between crystallographic native ligand and protein compared to the experimental results. The results ware ten conformations of 28P native ligand to LTA4H binding sites with different RMSD values and binding energies. The selected conformation was the conformation with the lowest RMSD value and met the validation requirements (RMSD ≤ 3.0 Å). In this study, the RMSD value was 2.08 Å in conformation 3 (Table 1).

### Docking quercetin on LTA4H

The optimized quercetin and bestatin were then docked to the LTA4H using the same grid box size during validation. The docking results showed ten conformations of quercetin and LTA4H. The conformation was selected by the lowest binding energy indicating the most stable conformation. The binding energy of 28P native ligand, quercetin, and bestatin to LTA4H was -10.22 kcal/mol (Table 1), -9.57 kcal/mol (Table 2), and -9.10 kcal/mol (Table 3), respectively.

**Table 2.** Docking result of quercetin on LTA4H

| Protein | Ligand | Conformation | Binding Energy (kcal/mol) | Amino acid residue | Groups in hydrogen bonds |
|---|---|---|---|---|---|
| LTA4H | Quercetin | 1 | -9.08 | - | - |
| | | 2 | -8.72 | - | - |
| | | 3 | -8.90 | ALA1137 | HN-O |
| | | 4 | -8.96 | - | - |
| | | 5 | -9.11 | - | - |
| | | 6* | -9.57 | ALA1137 | HN-O |
| | | 7 | -8.92 | - | - |
| | | 8 | -9.14 | ALA1137 | HN-O |
| | | 9 | -8.50 | - | - |
| | | 10 | -9.13 | ALA1137 | HN-O |

**Table 3.** Docking result of control bestatin on LTA4H

| Protein | Ligand | Conformation | Binding Energy (kcal/mol) | Amino acid residue | Groups in hydrogen bonds |
|---|---|---|---|---|---|
| LTA4H | Bestatin | 1 | -6.60 | - | - |
| | | 2 | -6.93 | GLY1268 | HN-O |
| | | 3* | -9.10 | GLY1269 | HN-O |
| | | 4 | -6.11 | - | - |
| | | 5 | -6.78 | - | - |
| | | 6 | -5.76 | - | - |
| | | 7 | -7.61 | - | - |
| | | 8 | -8.40 | GLY1269<br>HIS1295<br>HIS1299<br>TYR1383 | HN-O<br>HE2-O<br>HE2-O<br>HH-O |
| | | 9 | -7.66 | - | - |
| | | 10 | -9.10 | ALA1137 | HN-OXT |

Quercetin and bestatin interacted with LTA4H by hydrogen bonding interaction. Quercetin formed hydrogen bonding with LTA4H through ALA1137 residue, while hydrogen bonding of bestatin and LTA4H involved GLY1268 residue (Figure 3).

## Discussion

Quercetin has the potential as an anti-colorectal cancer therapeutic agent developed by the *in silico*. The energy binding of quercetin to LTA4H protein was -9.57 kcal/mol, while 28P native ligand and bestatin were -10.22 kcal/mol and -9.10 kcal/mol, respectively.

The *in silico* study of quercetin with caspase 3 and COX-2 proteins revealed the binding energy were -9.54 kcal/mol and -4.59 kcal/mol [13]. Another study showed the *in silico* study of bullatalicin to LTA4H (PDB ID: 3U9W) with grid sizes x = 60 Å, y = 60 Å, and z = 60 Å produced Gibbs free energy of -11.80 kcal/mol, while 28P native ligand -11.30 kcal/mol [14]. Other compounds derived from

*Lycopersicon esculentum* such as chlorogenic acid, gallic acid, protocatechuic acid, quercetin, and vanillic acid showed inhibitory activity against beta-catenin associated with colorectal cancer. By using HEX software and a beta-catenin target protein (PDB ID 1JDH) the binding energies of chlorogenic acid, gallic acid, protocatechuic acid, quercetin, and vanillic acid were -243.48, -183.25, -173.62, -255.21, and -170.22 kJ/mol, respectively [8].

An *in vitro* study showed that quercetin and luteolin had inhibition activity to HCT116 colorectal cancer cells by inducing apoptosis determined by Annexin V-FITC/PI [10]. LTA4H is also known as a target for 6-gingerol on aminopeptidase activity using the *p*-nitroanilide derivative of alanine (Ala-p-NA) as a substrate. The data showed that 6-gingerol treatment significantly inhibited HCT116 cell growth at 100 µmol/L. These findings support the evidence that quercetin has colorectal anticancer activity by inhibiting LTA4H which could be useful as a pharmaceutical active compound.

## Conclusion

These results indicate the lower binding energy of quercetin compared to bestatin as positive control. Based on *in silico* study, quercetin has potential as an colorectal anticancer agent by inhibiting LTA4H protein.

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## Declaration of interest

The authors declare no conflict of interest.

## Author contributions

MAWS and NPLL conceptualized the study design, MAWS and AAIRM investigated the data, MAWS, AAIRM, NKSA, and WNEP wrote original draft, MAWS, WNEP, NPLL reviewed and edited final version, MAWS looked for the funding, NPLL supervised all experiments. All authors have read the final manuscript.

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## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71: 209–249. doi:10.3322/caac.21660
2. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. pg. 2018; doi:10.5114/pg.2018.81072
3. Chen X, Wang S, Wu N, Yang CS. Leukotriene A4 hydrolase as a target for cancer prevention and therapy. Curr Cancer Drug Targets. 2004;4: 267–283. doi:10.2174/1568009043333041
4. Zhao S, Yao K, Li D, Liu K, Jin G, Yan M, et al. Inhibition of LTA4H by bestatin in human and mouse colorectal cancer. EBioMedicine. 2019;44: 361–374. doi:10.1016/j.ebiom.2019.05.008
5. Low CM, Akthar S, Patel DF, Löser S, Wong C-T, Jackson PL, et al. The development of novel LTA4H modulators to selectively target LTB4 generation. Sci Rep. 2017;7: 44449. doi:10.1038/srep44449
6. Wang L, Wang C, Jia Y, Liu Z, Shu X, Liu K. Resveratrol Increases Anti-Proliferative Activity of Bestatin Through Downregulating P-Glycoprotein Expression Via Inhibiting PI3K/Akt/mTOR Pathway in K562/ADR Cells. J Cell Biochem. 2016;117: 1233–1239. doi:10.1002/jcb.25407
7. Hirayama Y, Sakamaki S, Takayanagi N, Tsuji Y, Sagawa T, Chiba H, et al. [Chemotherapy with ubenimex corresponding to patient age and organ disorder for 18 cases of acute myelogeneous leukemia in elderly patients--effects, complications and long-term survival]. Gan To Kagaku Ryoho. 2003;30: 1113–1118.
8. Selvaraj J, Vishnupriya V, Sardar H, Balakrishna JP, Rex J, Mohan SK, et al. Molecular docking analysis of beta-catenin with compounds derived from Lycopersicon esculentum. Bioinformation. 2020;16: 801–806 doi:10.6026/97320630016801
9. Mathew NS, Kurrey NK, Bettadaiah BK, Negi PS. Anti-proliferative activity of Ensete superbum Roxb. Cheesman extract and its active principles on human colorectal cancer cell lines. J Food Sci. 2021;86: 5026–5040. doi:10.1111/1750-3841.15927
10. Chen Y, Hao E, Zhang F, Du Z, Xie J, Chen F, et al. Identifying Active Compounds and Mechanism of Camellia nitidissima Chi on Anti-Colon Cancer by Network Pharmacology and Experimental Validation. Evid Based Complement Alternat Med. 2021;2021: 7169211. doi:10.1155/2021/7169211

11. Kusuma AW, Nurulita NA, Hartanti D. Efek sitotoksik dan antiproliferatif kuersetin pada sel kanker kolon WiDr. PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia). 2010;
12. Jain AN, Nicholls A. Recommendations for evaluation of computational methods. J Comput Aided Mol Des. 2008;22: 133–139. doi:10.1007/s10822-008-9196-5
13. Putri PVP, Susanti NMP, Laksmiani NPL. Senyawa kuersetin sebagai agen antikanker kolorektal secara in silico. Jurnal Kimia. 2019; 166. doi:10.24843/JCHEM.2019.v13.i02.p07
14. Noviardi H, Fachrurrazie F. Potensi senyawa bullatalisin sebagai inhibitor protein leukotrien a4 hidrolase pada kanker kolon secara in silico. JF. 2015;5: 65–73. doi:10.33751/jf.v5i2.410