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# Interaction of Hypoxia-inducible Factor-1 with its Inhibitors Ursolic Acid Derivatives: A Molecular Docking Study

Jing Zou[1], Lixiao Wu[1], Li Yue[1,2], Pingxiao Xie[1,3], Chuanze Cai[1,4], Yuanxiao Xu[1,4], Jing Huang[1,2,3,*]

[1] College of Environmental and Biological Engineering, Putian University, Putian 351100, China

[2] Fujian Provincial Key Laboratory of Ecology-Toxicological Effects & Control for Emerging Contaminants, Putian 351100, China

[3] Key Laboratory of Ecological Environment and Information Atlas Fujian Provincial University, Putian 351100, China

[4] Department of Biotechnology, Putian Qingying New Energy Technology Co., Ltd. Putian, Fujian 351100, China

[*]jing_huang89@sina.com

## Abstract

**"HIF-1α pathway" is the main metabolism route in tissue in the condition of hypoxia. Ursolic acid derivatives inhibited the activities of HIF-1α and then alleviate the cancer. The previous experimental researches showed that ursolic acid derivative compound 11b had great biochemical toxicity to HIF-1α. This research found compound 11b hydrogen bonded to Phe572 residues and had strong electrostatic interaction with Asp569 and Asp571 residues. The hydrophobic interaction between compound 11b and Leu574 was also discovered by molecular docking study. The potential drug molecule compound 11b was indicated, as ursolic acid derivative compound 11b had strong compatibility with HIF-1α and had large biochemical toxicity to HIF-1α.**

## Keywords

**Molecular Docking; Intermolecular Interaction; Potential Drug Molecule.**

## 1. Introduction

Tumor is one of the most serious diseases in the human body. At present, most of the advanced malignant tumors were basically unable to be cured. The development of efficient new anti-cancer drugs has been the research focus of clinical medicine and pharmaceutical researchers. Malignant tumor cells usually proliferate in a hypoxic environment and mainly metabolize[1,2] through the "HIF-1 pathway". Therefore, the hypoxia-inducible factor HIF-1 was an important anti-tumor molecular target in mammals (including human body).HIF-1 was a heterodimer, including two subunits of HIF-1, based on O2 concentration expression, and a structurally expressed HIF-1 expression.HIF-1 was continuously synthesized under high O2 content. HIF-1 synthesis was inhibited under hypoxic conditions[3].

Over the past 20 years, scientists have worked to cure and alleviate cancer by blocking the malignant tumor HIF-1 pathway[4-7].The key to block the "HIF-1 pathway" of malignancy was to inhibit HIF-1 activity, On the one hand, HIF-1 can be made inactive inby hydroxylation modification of important histidine on the HIF-1 polypeptide chain (such as HIF-1 polypeptide chain) by some HIF-1 enzyme

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proteins (mainly HIF-1 hydroxylase) [4] ; On the other hand, the occurrence of the "HIF-1 pathway" was inhibited by the drug introduction of the corresponding inhibitory compound (inhibitor) acting on the different stages of the "HIF-1 pathway" [5,6]; Therefore, we should understand and explore the molecular design and mechanism of action of the corresponding inhibitory enzymes or inhibitors on HIF-1 targeted molecules, It was of great significance to the research and development of efficient new cancer drugs, enzyme protein engineering or biological engineering to achieve cancer cure.

Due to the importance of the "HIF-1 pathway" in tumor cells for the normal metabolism of tumor cells under hypoxia, many active component compounds of anticancer drugs were based on the inhibition of HIF-1 activity to achieve the anti-cancer effect [8].These inhibitors included several small molecule compounds, steroids, polypeptide chains as well as derivatives of some natural products. As the potential drugs, firstly, the compound can effectively bind to the active disease targeted protein site; secondly, the compound was biotoxicologic to the target protein, inhibiting the activity of the target protein; thirdly, the physical and chemical properties of the compound, body absorption, metabolism, toxicity and other series of key factors (ADME / Tox) met the medicinal requirements. [10] The tyrosine kinase inhibitors Herceptin, ZD1839, and Glivec was identified in the course of the study. The MEK inhibitor, PD98059, The Pi3 K inhibitor, LY294002, The PKC inhibitor, calphostin C, Both the MAPK inhibitors PD98059 and U0126 and the m-TOR inhibitor rapamycin can inhibit the associated signal transduction pathways, Regulating the transcription, translation, or degradation process of HIF-1, causing reduced intracellular HIF-1 levels. when the novel topoisomerase (Top2) inhibitor MFTZ-1 was significantly lower than its Top2 inhibitory concentration, It can have a significant inhibitory effect on HIF-1 under complex conditions, including hypoxia, normogen, and growth factor induction. [10].

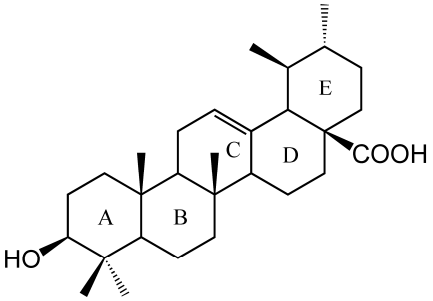


**Figure 1.** Molecular structure of Urinonic acid

The molecular structure of urllic acid was shown in Figure 1, belonging to pentacyclic terterpenoids. It was widely found in the leaves and fruits of various natural plants, such as loquat leaves, hawthorn, plantain and other natural products contain a large amount of urllic acid, mainly in the form of free or binding with polysaccharides. Urinic acid has strong biological activity, natural antioxidant activity, anti-inflammatory and anti-blood lipid activity and anti-tumor activity. and it was an important active ingredient of food antioxidant, anti-inflammatory and liver-preserving drugs and anti-cancer drugs[11].Because the application of ursolic acid in anticancer drugs was limited to the large concentration of ursolic acid, the development of ursolic-acid-related anticancer drugs is greatly limited. In recent years, the anticancer drugs of ursolic acid have been studied mainly by modifying the natural ursolic acid structure to reduce the drug use concentration. Lin et al. modified -OH and -COOH of ursolic acid and found compound[12] to inhibit hepatoma cells; Chi et al. designed and synthesized a series of ursolic acid and its derivatives (Figure 2) to explore the biotoxicity of ursolic acid and its derivatives on HIF-1 protein at the molecular scale and found that some of the compounds had good HIF-1 inhibitory active[13].Among them, the 50% inhibitory concentration of compound 11b was 36.9 μmol/L, that is, 36.9 μmol/L compound 11b could inhibit HIF-1 synthesis by 50%, with strong biotoxicity to HIF-1 and strong ability to induce apoptosis in cancer cells,[14].In this study,

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based on the affinity of compound 11b and the HIF-1 protein, the pharmacogenesis of compound 11b was investigated from a theoretical perspective.

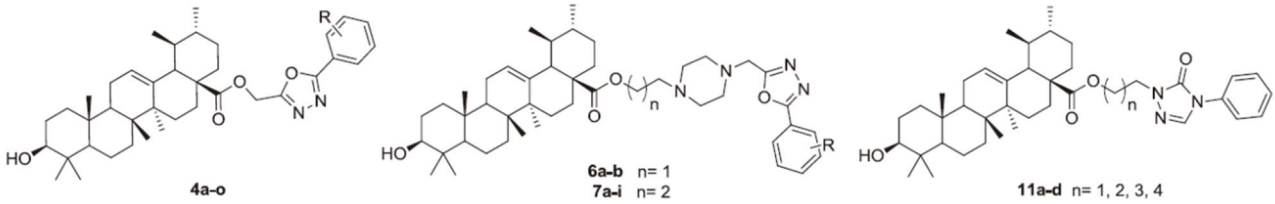


**Figure 2.** Ursolic acid derivative acting on HIF-1 molecular targets designed by Chi et al[13]

## 2. Methods

In this study, molecular docking technology was mainly used to determine the fit degree of ursolic acid derivatives and HIF-1 molecular targets. Molecular docking (molecular docking) technology was employed to simulate the interaction of between small-molecule ligand and receptor biological macromolecules based on the lock-key principle (lock and key principle) of ligand-receptor action[14,15].At present, molecular docking can effectively match the corresponding small molecule compounds according to the structure and charge distribution properties of molecular targets, which has been widely used in database search in structure-based drug design.[14,15].

### 2.1 System Preparation

Three-dimensional crystal structure coordinates of the hypoxia-oxygen-inducing factor HIF-1 molecular target (PDB number: 1LM8[16]) can be obtained from the RCSB protein crystal database.1LM8 structure contained four parts including HIF-1 protein, elongin B antigen, elongin C antigenas well as the tumor suppressor pVHL.The 1LM8 structure was edited in the Maestro program package[17] by removing the coordinates of the elongin B antigen, the elongin C antigen, and the tumor suppressor pVHL, as well as the crystal water on the HIF-1 protein.The treated HIF-1 protein structure and some of its important structures are shown in Figure 3. The missing amino acid sequences of the Asp556-Leu557-Asp558-Leu559-Glu560 in the HIF-1 protein were complemented by using the Modeller 9.21 program[18].The OPLS2001 force field was used to set the molecular dynamics parameters of each atoms in the Maestro package[17] to complement the missing hydrogen atoms found in the protein crystal. Molecular dynamics optimization was performed using the OPLS2001 force field with default 0.3A RMSD. The structure of ligand compound 11b was constructed by Chemdraw14.0 software and stored in SDF format and HIF-1a crystal structure (PDB Code: 1LM8) was optimized by Discovery studio (DS3.0) by deleting non-essential protein structure, retaining the required protein structure HIF-1, defining the entire protein as active center, determining active region coordinates, and saving in PDB format.

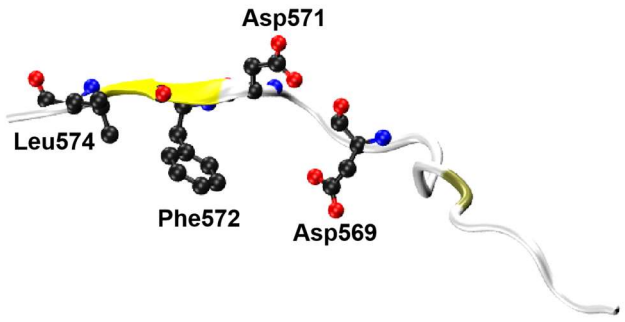


**Figure 3.** Crystal structure of HIF-1 protein and some important amino acids

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### 2.2 Molecular Docking

The entire molecular docking process was carried out at the Maestro interface [17] with high-precision molecular docking of HIF-1 proteins using the glide module[19]. Lattice parameters were set for the prepared HIF-1 protein.The default lattice (20 × 20 × 20Å3) was used and the lattice generation parameters and molecular docking process parameters were set as default. The whole docking process included protein preparation, receptor grid generation and ligand docking. Finally, according to the binding site, the network file was generated. other parameters was default set following by the docking and evaluating.

## 3. Results and Discussion

The interaction force formed by the target protein HIF-1 with the compound 11b molecule is shown in Figure 4. Virtual screening of drugs was achieved via the binding pattern and affinity between HIF-1 protein and compound 11b. The central coordinates of the active region box were located in the coordinate of (X: 24.70, Y: 12.87, Z: 134.30) with the docking score of -1.100, indicating the good intermolecular fit between the target protein HIF-1 and compound 11b. The molecular interaction between proteins and small molecule compounds mainly contained hydrogen bond interaction, electrostatic interaction and hydrophobic interaction.

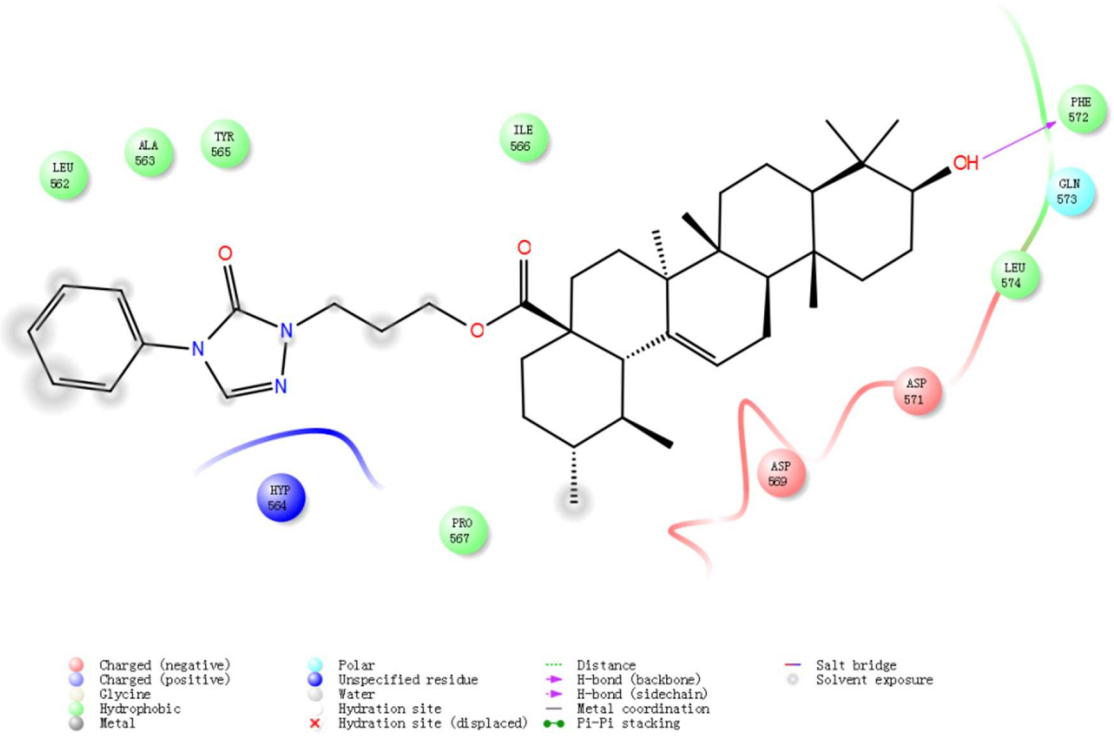


**Figure 4.** Amino acids acting with compound 11b in the H I F-1 protein

The interactions on the spatial structure of the protein (HIF-1) and the small molecule compound 11b with the surrounding amino acids are shown in Figure 5. The hydrogen atoms on the hydroxyl group on compound 11b was 2.60 Å apart from the base oxygen atoms on HIF-1. The hydroxyl group on compound 11b was capable of hydrogen bond interaction with the HIF-1 protein Phe572 amino acid. There were also electrostatic interactions between the pentocyclic trterpene structure on compound 11b and Asp569/Asp571 amino acids, and hydrophobic interactions with Leu574 amino acids. Overall, it was found that compound 11b and HIF-1 protein can interact strongly by molecular docking simulations, and can become potential drug molecules, which can continue to be used for subsequent pharmacokinetic test analysis.

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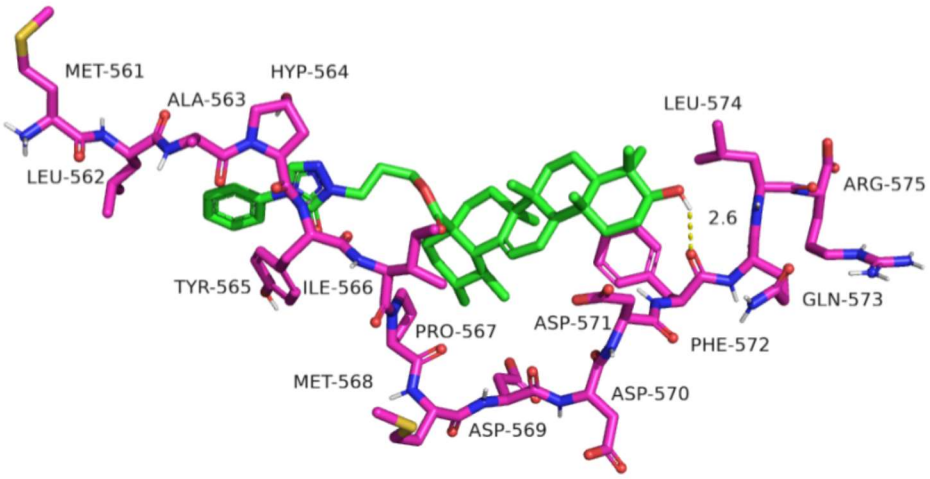


**Figure 5.** Interactions on the spatial structure of the H I F-1 protein and compound 11b, and the arrangement of the surrounding amino acids

## 4. Conclusion

Uronic acid and its derivatives have strong anti-tumor activity and this study further explored the fit matching of HIF-1 protein targeting molecules with uronic acid derivative compound 11b based on previous studies of biotoxicology effects. Compound 11b was found to have a strong interaction with HIF-1 by molecular docking techniques, and can be identified as a potential drug molecule.

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