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A bufadienolide derived androgen receptor antagonist with inhibitory activities against prostate cancer cells

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ABSTRACT

Molecular docking studies have shown that $\Delta^{8,14}$-anhydrobufalin (**1**) exhibited more potent binding affinity on androgen receptor (AR) than $\Delta^{14,15}$-anhydrobufalin (**2**) and bufalin (**3**). To validate the docking results, compounds **1** and **2** were synthesized. The AR competitive binding assay indicated that the IC_{50} values of **1**–**3** were 1.9, >50 and >50 μM (relative binding affinity), respectively, which confirmed that our theoretical binding mode was reliable and predictable. Furthermore, compound **1** was found to show more potent inhibitory activity against the androgen dependent LNCaP cancer cells than the androgen independent PC3 cancer cells, but exhibited less inhibition on the Na^+/K^+ ATPase as compared with the parent compound **3**. To the best of our knowledge, compound **1** represented the first AR antagonist derived from bufadienolide discovered through a series of combined approaches of molecular docking and actual experimental validation.

1. Introduction

Androgen receptor (AR) belongs to the steroid nuclear receptor super-family [1]. It is activated by endogenous androgens as testosterone and 5a-dihydrotestosterone (DHT) or exogenous compounds, and regulates genes for male differentiation and development [2]. However, high levels of AR expression may lead to severe diseases like prostate cancer (PCa). Recently, AR was found to play a critical role in PCa since approximately 80–90% of PCa are androgen dependent at initial diagnosis [3,4]. Thus it has become an attractive target for the treatment of PCa. Although several pure AR antagonists, such as bicalutamide and flutamide, were developed for PCa therapy, they could not completely blocks binding of DHT to AR and the small amount of free DHT still stimulate prostate cancer growth [5]. Furthermore, bicalutamide can exhibit some agonist activity in cells containing mutant AR [6]. Thus, a sustained effort for the development of new and more effective AR antagonist has been undertaken.

Bufalin (**3**, Scheme 1), a typical bufadienolide, has been reported to show potent antineoplastic activity against human prostate cancer cells LNCaP and DU145 [7]. However, it was reported to be five times more lethal than ouabain due to its much stronger inhibition on Na^+/K^+-ATPase [8], which greatly hindered the clinical application [9].

Close examination of the structure of **3** revealed the similarity to those of steroidal AR antagonists, such as VN/85-1 [10]. Both of them possessed a steroidal skeleton with an unsaturated substitution at C-17. However, the steroid moiety of bufalin is saturated in contrast to the presence of at least one double bond in the steroidal AR antagonists. We hypothesized that introduction of a double bond in bufalin would increase the interactions with androgen receptor.

In order to test the hypothesis, firstly, we virtually introduced a double bond around the hydroxyl group at C-14 of **3** considering that it was in the middle of the molecule and important for the conformation of the whole molecule. Then, the molecular docking method was used to compare the interactions of **3** and the two derivatives, i.e., $\Delta^{8,14}$-anhydrobufalin (**1**) and $\Delta^{14,15}$-anhydrobufalin (**2**, Scheme 1) with androgen receptor. Finally, the actual derivatives were synthesized and their activities toward the two molecular targets were tested.

2. Materials and methods

2.1. Molecular docking study of compounds 1–3 to AR

2.1.1. Homology modeling

The 3D model of the androgen receptor (AR) in its inactive form was constructed based on the known antagonist form of human glucocorticoid receptor (PDB ID: 3H52) [11], a homologous protein

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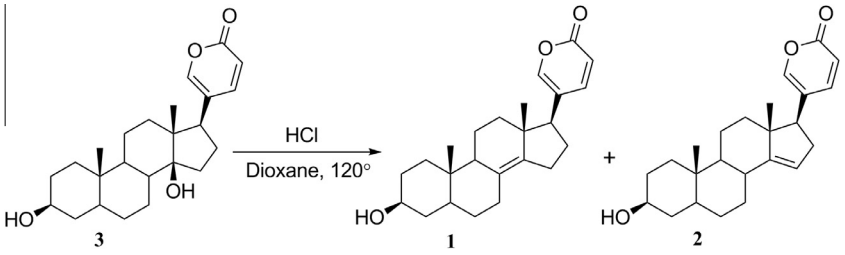

Scheme 1. Structure formulae of compounds **1**–**3**.

of AR (they share >50% sequence similarity) and an agonist form of AR (PDB ID: 2PIT) [12]. Sequence alignment and homology modeling were performed using Modeller V9.10 [13]. The final sequence alignment was visualized using ESpript [14] as shown in Fig. 1.

2.1.2. Molecular dynamic (MD) simulation

In order to obtain a more stable conformation of AR in solution, MD simulation was performed. Simulations were conducted with the OPLS all-atom force-field implemented in GROMACS 4.5.3 [15]. Topology files were generated using pdb2gmx module in Gromacs, then this system was solvated by water suing TIP4P water model in a cubic box extending 10 Å around the receptor. In addition, the system was neutralized using sodium chloride and the concentration was adjusted to 0.17 mM by genion (in Gromacs). Long-range electrostatic interactions were treated using the particle-mesh Ewald method [16]. Periodic boundary conditions were applied to avoid edge effects in all calculations. The temperature was kept constant at 300 K by separately coupling the water, ions, and protein in a thermal bath using the Berendsen thermostat method [17] with a coupling time of 2 ps. Berendsen pressure coupling was used for the equilibration of the systems. The solvated system was underwent two energy minimizations with protein position constrained and none restrain at all. Following, energy minimized solvated system was equilibrated by 100 ps protein position restrained NVT and NPT process at 300 K. Finally, a 10 ns NPT equilibration was conducted without restriction. After 10 ns equilibration, the final conformation was extracted and used for docking.

2.1.3. Molecular docking

2.1.3.1. Protein preparation and grid generation. Protein structure was prepared with Protein Preparation Wizard [18] in Maestro 9.0, energy was minimized using OPLS force-field and default setting. The A chain of antagonist human glucocorticoid receptor was overlapped into above mentioned structure, and the position of co-crystallized ligand was used as a reference for next step. Grid was generated by Glide 5.5 using default setting.

2.1.3.2. Ligand preparation. Ligand was drawn using Maestro 9.0 [19] and prepared using LigPrep [20] application. MMFFs force-field was chosen. Other parameters use default values.

2.1.3.3. Docking procedure. Molecular docking was performed using Induced Fit Docking (IFD) [21] and Rigid Docking [22] modules in Maestro 9.0 (SP mode). The ligand flexibility was considered in both approaches. Grid box was centered on R752, R711, L704 and W741, which was based on crystal structure of AR bound to DTH (PDB ID: 2PIT) [12]. Since the binding site of AR is a very large binding cavity, we extend the outer box length to 24.6 Å (from 18.7 Å). Flexible residues were defined using residues within 5.0 Å distance from the reference ligand for the Induced Fit Docking method, while for the Rigid Docking method, all residues were kept rigid. Best pose of each molecule was extracted, and final result was visualized using PyMol 1.3 [23] and LigPlot [24].

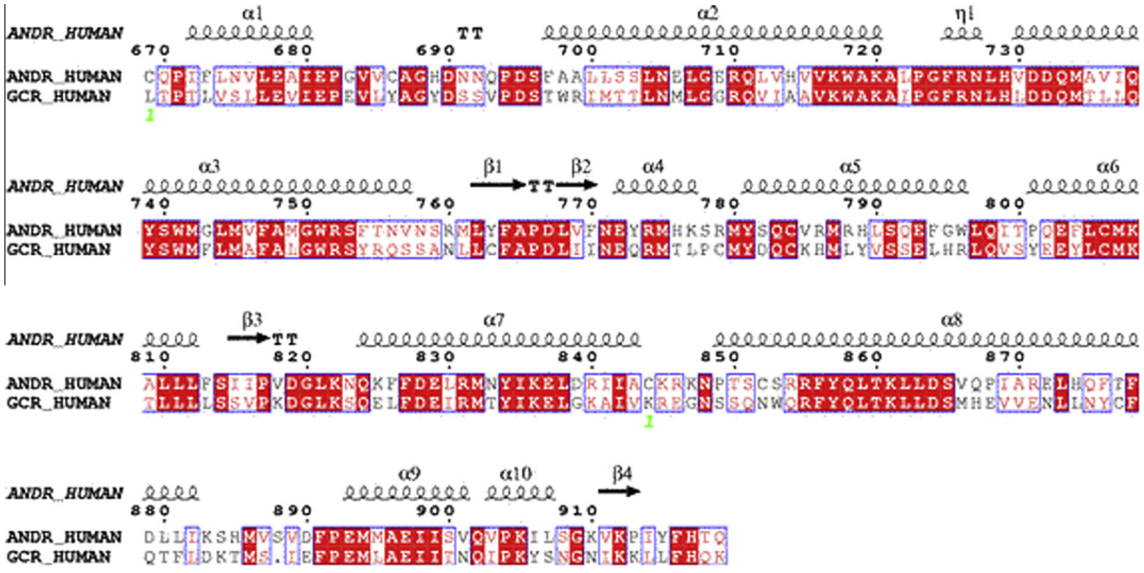

Fig. 1. Sequence alignment was performed in Modeller 9.10 and drawn with ESPript. The sequence of androgen receptor (PDB code: 2PIT) was shown on the upper side and the sequence of glucocorticoid receptor (PDB code: 3H52) was shown downside.