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Design and synthesis of novel androgen receptor antagonists via molecular modeling

Chao Zhao, You Hee Choi, Daulat Bikram Khadka, Yifeng Jin, Kwang-Youl Lee *, Won-Jea Cho *

College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Republic of Korea

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ABSTRACT

Several androgen receptor (AR) antagonists are clinically prescribed to treat prostate cancer. Unfortunately, many patients become resistant to the existing AR antagonists. To overcome this, a novel AR antagonist candidate called DIMN was discovered by our research group in 2013. In order to develop compounds with improved potency, we designed novel DIMN derivatives based on a docking study and substituted carbons with heteroatom moieties. Encouraging in vitro results for compounds **1b**, **1c**, **1e**, **3c**, and **4c** proved that the new design was successful. Among the newly synthesized compounds, **1e** exhibited the strongest inhibitory effect on LNCaP cell growth (IC_{50} = 0.35 μM) and also acted as a competitive AR antagonist with selectivity over the estrogen receptor (ER) and the glucocorticoid receptor (GR). A docking study of compound **1e** fully supported these biological results. Compound **1e** is considered to be a novel, potent and AR-specific antagonist for treating prostate cancer. Thus, our study successfully applied molecular modeling and bioisosteric replacement for hit optimization. The methods here provide a guide for future development of drug candidates through structure-based drug discovery and chemical modifications.

1. Introduction

The androgen receptor (AR) belongs to the nuclear receptor family of ligand-activated transcriptional factors. Testosterone and dihydrotestosterone (DHT) are the most common ligands of AR.[1–3] Since the AR plays a critical role in prostate cancer, many AR antagonists have been used clinically to treat prostate cancer.[4,5] However, long term treatment of AR antagonists often leads to drug-resistance and mutations in the AR receptor are considered to be the main reason.[6–10] As reported, AR antagonists prevent formation of the activation function 2 (AF2) site essential for recruitment of AR coactivators by displacing helix 12 (H12).[11] Mutations in AR usually allow H12 to reposition to form AF2 again even in the presence of an antagonist.[12] For example, the first nonsteroidal AR antagonist flutamide (Fig. 1) acts as an agonist when the T877A mutation is present in AR.[13]

Several of the AR antagonists administered clinically are derivatives of flutamide.[14,15] A novel AR antagonist enzalutamide (MDV3100) was approved by the U.S. Food and Drug Administration (FDA) in 2012. The drug was investigated based on the research of flutamide.[16] Due to the structural similarity, flutamide-like drugs are expected to possess similar physicochemical and pharmacological limitations as flutamide. Thus, considering this fact, more and more research groups have focused on the development of AR antagonists with a novel scaffold to treat prostate cancer.[17–19]

Our research group has a long-standing interest in discovery of novel AR antagonists for treating prostate cancer. In a previous study, we successfully discovered nicotinamide derivative 6-(3,4-dihydro-1*H*-isoquinolin-2-yl)-*N*-(6-methylpyridin-2-yl) nicotinamide (DIMN) (Fig. 1) as a novel class of nonsteroidal AR antagonist for the treatment of prostate cancer.[20,21] DIMN was anticipated to interact with AR as shown in Figure 2. On one side of the ligand-binding domain (LBD), the amide group and N of pyridine of DIMN form several hydrogen bonds with Arg752, Phe764, and Gln711 of AR (Fig. 2B). On the other end, the isoquinoline ring approaches Met895 of H12 and possibly pushes the helix out of the LBD to attain an open conformation of AR.

AR perfectly accommodates linear DIMN. However, a large space of the hydrophobic cone-shaped cavity surrounded by Trp741, Met742, His874, Met895, Ile898, Ile899, and Val903 above the isoquinoline ring remains unoccupied (Fig. 2A). Thus, we hypothesized that introduction of a long linear hydrophobic group at the end of the isoquinoline ring, which can fit into this cavity, may enhance binding affinity and increase displacement of H12.

* Corresponding authors. Tel.: +82 62 530 2939; fax: +82 62 530 2911 (K.-Y.L.); tel.: +82 62 530 2933; fax: +82 62 530 2911 (W.-J.C.).
E-mail addresses: kwanglee@jnu.ac.kr (K.-Y. Lee), wjcho@jnu.ac.kr (W.-J. Cho).

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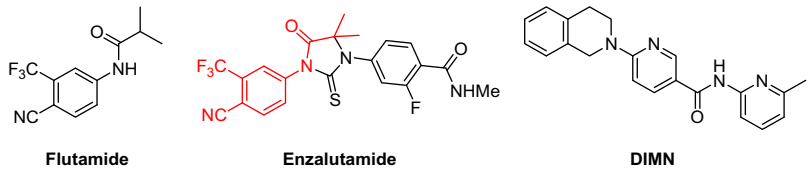

Figure 1. Known AR antagonists.

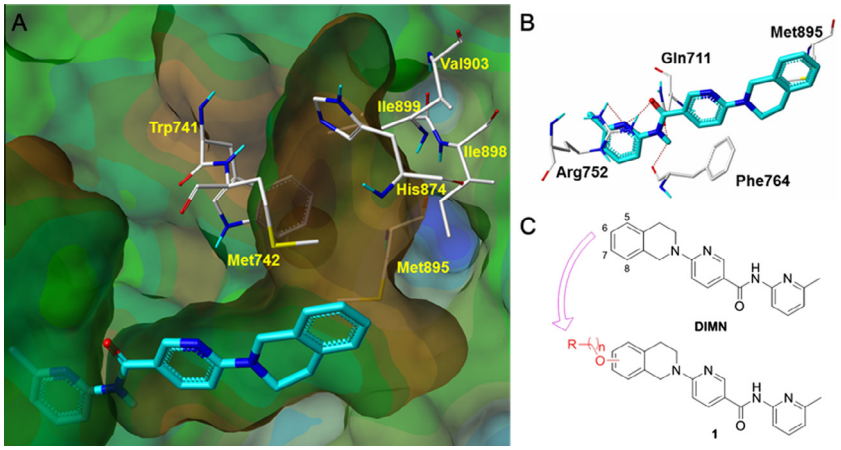

Figure 2. Design of novel derivatives of DIMN. (A) & (B) The binding mode of DIMN in complex with AR. Hydrogen bonds are represented as red dashed lines. Hydrophobicity (lipophilicity) is represented as textured colors where dark brown is more hydrophobic and bright green is less hydrophobic. (C) Design of new DIMN derivatives.

Therefore, novel derivatives of DIMN that contain an additional linear group on the isoquinoline ring were designed (Fig. 2C).

Several compounds were designed and applied to the docking study. Most of the newly designed compounds exhibited better docking scores than DIMN. Among the compounds subjected to docking, derivatives with an additional group at the 6-position of the isoquinoline ring exhibited the best docking results. The binding model of one newly designed compound, **1b**, is illustrated in Figure 3. The amide moiety of **1b** interacted with Arg752, Phe764, and Gln711 of AR via several hydrogen bonds. An additional hydrogen bond was formed between the ethereal O of the isoquinoline ring and Thr877. As expected, the *n*-Pr moiety occupied the hydrophobic cavity. These two new interactions may increase the binding affinity between the compound **1b** and the AR. With these encouraging docking results in hand, novel DIMN derivatives with linear alkyl substituents were synthesized.

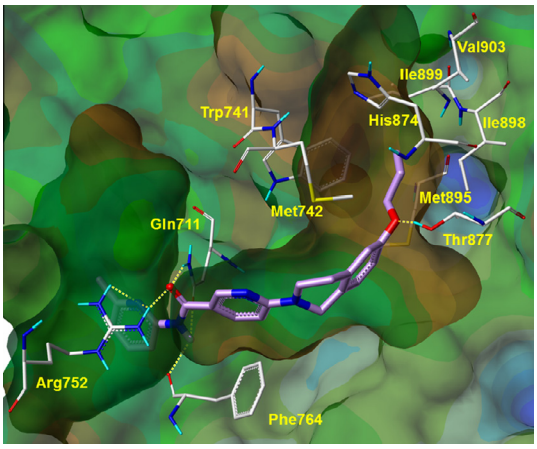

Figure 3. The binding mode of compound **1b** in complex with AR. Hydrogen bonds are represented as yellow dashed lines. Hydrophobicity (lipophilicity) is represented as textured colors where dark brown is more hydrophobic and bright green is less hydrophobic.

In addition to the development of DIMN derivatives with linear substituents, preparation of bioisosteres of DIMN was considered to be beneficial. Previous studies show that compounds with a nicotinamide moiety exhibited much greater activity than those with a benzamide functional group (**2**, Fig. 4). Coincidentally, another research group showed that replacing pyridinone with pyrimidinone improved activity.[22] On the basis of these findings, we hypothesized that an additional N in the core ring may improve bioactivity. Therefore, changing the pyridine ring to a pyrazine and a pyrimidine may gain bioisosteric effects and enhance hydrophilicity. For this reason, compounds **3a** and **4a** were designed and applied to the docking study (Fig. 4).

The docking study demonstrated that **3a** and **4a** had higher docking scores than those of DIMN. A complex of **3a** and AR showed that the amide moiety interacted with Phe764, Arg752, and Gln711 through five hydrogen bonds (Fig. 5A). The N of methyl pyridine associated with AR via hydrogen bonding to Arg752. The N from the pyrazine ring created another hydrogen bond with Gln711. This demonstrated that introducing an additional N to the pyrazine ring is beneficial. The isoquinoline ring faced Met895 to repel H12 and attain an open conformation. Compound **4a** also exhibited interactions with AR similar to DIMN (Fig. 5B). The amide moiety interacted with Phe764, Arg752, and Gln711 of AR via several hydrogen bonds. The isoquinoline ring was oriented in a position to push H12 further away from the ligand binding pocket. According to these results, several additional compounds belonging to these two new series of pyrazinamides and pyrimidinamides were synthesized.

2. Chemistry

The detailed synthesis procedure for nicotinamides **1a–h** is described in Scheme 1.[20] Chloronicotinic acid **5** was refluxed with