Structure-Based Virtual Screening and Identification of a Novel AR Antagonist,

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Supplementary File 1. Receptor-based virtual screening and lead identification. The FlexX docking program was applied as a molecular modeling tool. Computational calculations were performed by the Sybyl molecular modeling software, version 8.1.1, supplied by Tripos Associates, operating under Red Hat Linux 4.0 with an IBM computer (Intel Pentium 4, 2.8 GHz CPU, 1 GB memory). The structures of ligands were drawn in the Sybyl package with standard bond lengths and angles and minimized using the conjugate gradient method. The Gasteiger-Huckel charge with a distance-dependent dielectric function was applied for the minimization process. The docking simulations were carried out using FlexX Single Receptor mode with a Mol2 file molecule as a ligand source. After running FlexX, 54 docked conformers were displayed in a molecular spreadsheet to rank the scores.

Following preliminary tests against the AR, DIMN, which ranked first in the predicted scoring table, showed the strongest antagonistic activity, suggesting that our FlexX calculation is highly confident. However, potency does not always ensure the utility of a lead compound. To determine the potential of a possible lead compound, Lipinski's rule and the novelty of the chemical structure must be considered. Lipinski's rule is critical for drug development methods in which a lead is optimized for increased activity and selectivity or drug-like properties such as absorption and permeation (1). In general, a lead is more likely to be a successful drug candidate if it meets the following criteria of Lipinski: 1) no more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms); 2) no more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms); 3) a molecular mass not greater than 500 daltons; and 4) an octanol-water partition coefficient log *P* not greater than 5. In addition, a lead would be favored if the chemical structure of the lead is new and is easy to synthesize. Following the ranking of the virtual screen hits in terms of potency,

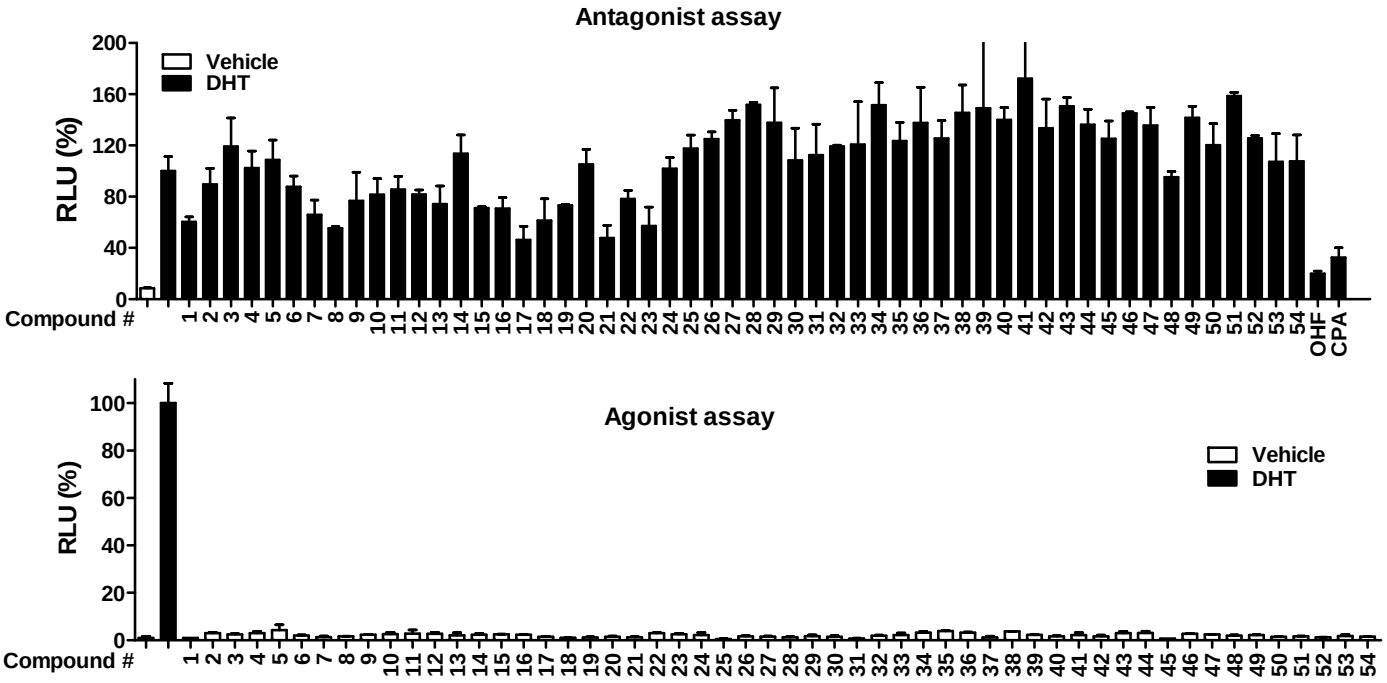
Lipinski's rule and the novelty of the chemical structure, DIMN was selected as a lead for a new generation of AR antagonists.

Supplementary Figure 1. The antagonistic/agonistic effects of virtual screening-selected compounds on the AR. AR transcriptional activity was determined in COS-7 cells transiently co-transfected with pcDNA3.AR (50 ng) and pARE$_2$-TATA-Luc (350 ng). After a 24-h transfection, cells were treated with 1 μM chemicals selected by virtual screening for AR antagonist in the presence (black bar) or absence (white bar) of 0.3 nM DHT for an additional 24 h.

Supplementary Figure 2. Hypothetical binding mode of DIMN into the AR. (**a**) Low-energy binding conformations of DIMN bound to WT AR. (**b**) Cross-section prediction of DIMN bound to WT AR LBD. Hydrophobicity is represented as textured colors. Brown is more hydrophobic and green is less hydrophobic. Hydrogen bonds are depicted as dotted lines. The hydrophobic isoquinoline is thoroughly enclosed by the hydrophobic residues of the AR LBD. The length of the hydrogen bond between Gln 711 and NH of the amide is 1.63 Å, and the distances between the Arg 752 side chain, the carbonyl group of the amide, and the nitrogen of pyridine are 1.81, 1.89, and 2.19 Å, respectively.

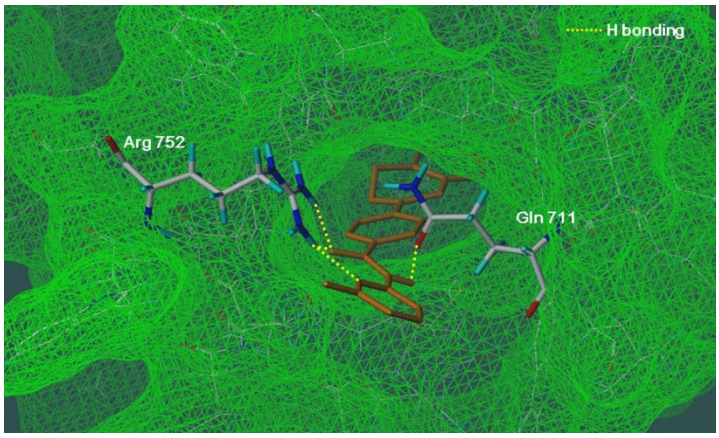
Supplementary Reference

1. Oprea, T. I., Davis, A. M., Teague, S. J., and Leeson, P. D. (2001) *Journal of chemical information and computer sciences* **41**, 1308-1315



Supplementary Figure 1

A



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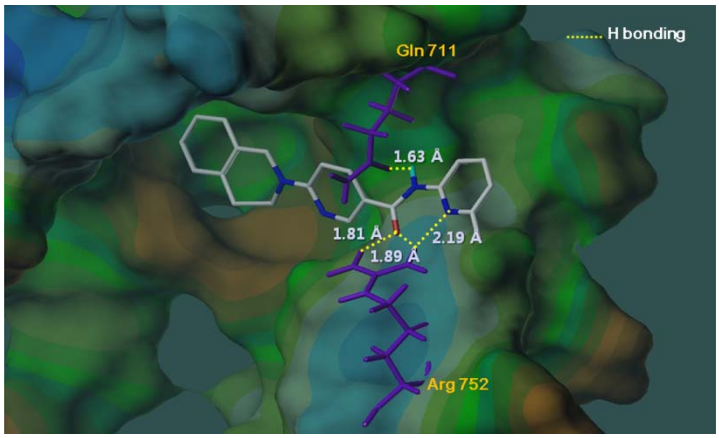

Supplementary Figure 2