**Supporting Information**

# Identification of triptophenolide from *Tripterygium wilfordii* as a pan-antagonist of androgen receptor

**Yang He[a], Meng Wu[a], Yangguang Liu[a], Quanjie Li [a], Xiaoyu Li [a], Laixing Hu [a*], Shan Cen[a *], Jinming Zhou[a *]**

Corresponding author: Jinming Zhou, zhoujinming@imb.pumc.edu.cn. Shan Cen, shancen@imb.pumc.edu.cn; Laixing Hu, hulaixing@ imb.pumc.edu.cn. Institute of Medicinal Biotechnology, Chinese Academy of Medical Science, Beijing, China 100050, Tel: 86-10-63131011; Fax: 86-10-63017302

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## Material and Methodology

### *Cell lines*

The LNCaP cell line was purchased from American Type Culture Collection (ATCC,CRL-1740), and cultured with RPMI-1640 supplemented with 10% Fetal Bovine Serum (GiBCO). The PC-3 cell line was kindly provided by Dr J.H.Wu (McGill University, Canada), and cultured with F-12K supplemented with 10% Fetal Bovine Serum.

### *MTT assay*

LNCaP cells were seeded at a density of 4-5×$10^3$ cells per well in 96-well plate in complete RPMI-1640 growth medium, or PC-3 cells in complete F-12K growth medium at the same density. After overnight incubation, 1 μL DMSO (Sigma-Aldrich) or 1 μL test compounds were added to each well at the designated concentration. After 72 h incubation, 20 μL of MTT (Invitrogen) solution (5 mg/mL in PBS) were added per well and incubated for another 2 h. The MTT formazan formed by metabolically viable cells was dissolved in 100 μL isopropanol. The absorbance was measured at 570 nm wavelength on a plate reader (EnSpire 2300, PerkinElmer). Experiments were performed in triplicate. The value of DMSO group was defined as 100%.

### *Real time PCR assay*

The LNCaP cells were seeded at a density of 3×$10^5$ cells per well in 6-well plate with phenol red-free RPMI-1640 medium containing 10% charcoal-stripped FBS for 24 h, and were then treated with 1nM of DHT 1 μL and 1 μL test compounds at the designated concentration for further 24 h. Cells were lysed with Trizol (Invitrogen), and separated with chloroform (Sigma-Aldrich). Isopropanol and 75% ethanol (Sigma-Aldrich) were used to extract mRNA. Then the *PSA* mRNA and *GAPDH* mRNA levels were tested with one step SYBR PrimeScript RT-PCR kit (TaKaRa) on the PCR instrument (Stratagene MX3000P, Agilent). $2^{-\Delta\Delta Ct}$ was used to quantified the *PSA* mRNA level relatively. All experiments were run in duplicate. The primer of PSA and GAPDH were listed as follows:

**Table S1.Primer sequences for real-time RT-PCR.**

| Segments | Primer sequences |
|---|---|
| PSA | up：5’-GGT GAC CAA GTT CAT GCT GTG-3’ |
| | down：5’-GTG TCC TTG ATC CAC TTC CG-3’ |
| GAPDH | up：5’- GGT ATC GTG GAA GGA CTC ATG AC -3’ |
| | down：5’- ATG CCA GTG AGC TTC CCG TTC AG -3’ |

### *Extraction of Tripterygium wilfordii root.*

The extracts of *Tripterygium wilfordii* root (100g) were heating reflux with ethanol in an oil bath for 2 h after being soaked overnight. Next, collected solution and added ethanol (1 L) to the residues for refluxing another two times. Then the collected solution was filtered and evaporated the

ethanol under low pressure.

***Dual-Luciferase reporter assay***

Plasmid F876L is full length cDNA of mutant AR that harbors F876L mutation. Plasmid T877A is full length cDNA of mutant AR that harbors T877A mutation. Plasmid W741C+T877A is full length cDNA of mutant AR that harbors W741C and T877A mutation. These plasmids F876L and W741C+T877A were kindly provided by Dr J.H. Wu (McGill University, Canada), and the plasmid T877A was constructed in house. During the reporter assays, 24 h before transfection, PC-3 cells were seeded at a density of 6-7×$10^4$ cells per well in 24-well plate and subsequently co-transfected with 100 ng of PSA-luc, 20 ng of F876L (20 ng T877A or 50 ng W741C+T877A), and 1 ng of Renilla plasmids using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's protocol. 24 h after transfection, the medium was changed to phenol red-free RPMI-1640 supplemented with 10% charcoal-stripped FBS, containing 1nM of DHT 1 μL and 1 μL test compounds at the designated concentration. After further 24 h, the cells were lysed in 100 μL per well passive lysis buffer, and 20 μL of the cell lysates was used for detection of the luciferase activity using Dual Luciferase Assay System (Promega) on a plate reader (Centro XS3 LB 960, Berthold). All experiments were run in triplicate.

***Western blot***

LNCaP cells were seeded at a density of 3×$10^5$ cells per well in 6-well plate. After overnight incubation, 1 μL DMSO (Sigma-Aldrich) or 1 μL triptophenolide were added to each well at the designated concentration. After another 24 h incubation, the cells were lysed with RIPA. Then the protein lysis buffer was treated with 10% SDS–PAGE for western blot analysis. The following antibodies were used for the detection of proteins: rabbit anti-AR (N-20, 1:500, Santa Cruz). Mouse anti-actin (1:5000, Abcam) was used as a loading control. Proteins were visualised using anti-mouse or anti-rabbit HRP-conjugated secondary antibodies (1:5000, Santa Cruz) and ECL-Plus (Millipore). The AR protein was semi-quantified through the ImageJ software.

***Immunofluorescence***

LNCaP cells were grown on the glass cell culture dish in phenol red-free RPMI-1640 medium containing 10% charcoal-stripped FBS for 24 h, and were then treated with DMSO, 1 nM DHT, 500 nM enzalutamide and 1 nM DHT, 5 μM triptophenolide and 1 nM DHT, respectively for further 24 h. Cells were fixed with 4% (vol/vol) paraformaldehyde and permeabilized with 0.2% Triton X-100. Then the cells were incubated with AR antibodies (N-20, 1:100, Santa Cruz). The secondary antibodies, goat anti-rabbit with FITC (Santa Cruz) at 1:100 were used. The counterstain DAPI was used to visualize cell nucleus. The images were detected under UltraVIEW vox spinning disc confocal scanning system (Perkin Elmer) on a Olympus IX81 microscope.

***Ligand binding assay***

The binding affinity of triptophenolide to AR-LBD protein *in vitro*, relative to dihydro-testosterone (3.6 nM Fluormone DHT Green at final concentration),

was evaluated by AR fluorescence polarization (FP) assay, using Polar-Screen AR competitor assay kit (P3018, Invitrogen ) on a plate reader ( VICTOR X5, PerkinElmer ) .

***Molecular Modeling***

The three-dimensional structures of triptopheolide and other antiandrogens were generated using the “Prepare Ligands” module implemented in Discovery Studio. Using crystal structures of AR-LBD/DHT (PDB entry: 1t65) and ERα-LBD/hydroxytamoxifen (PDB entry: 3ert ) as templates, 50 structural models of antagonistic AR-LBD were generated using the “Protein Modeling” module in Discovery Studio. The structural model with lowest objective function was selected for further study. The selected model, like ERα-LBD/hydroxytamoxifen, has its H12 at the coactivator-binding groove. The binding mode for compounds towards the binding site of the homology model was generated through molecular docking using MOE ( Molecular Operating Environment ) version 2009.10. In general, the docking was performed through the ‘DOCK’ module in MOE using the Alpha-Triangle placement method. Refinement of the docked poses was carried out using the Forcefield refinement scheme and scored using both the affinity dG and London dG scoring system. The pose with the highest docking score was returned for further analysis.

**NMR, MS, and HPLC analyses data**

**Table S2. Purity and Peak Attributions by HPLC analysis.**

| Compound | Molecular Formula | Purity (%) | Retention Time (min) | Method |
|---|---|---|---|---|
| Triptophenolide | $C_{20}H_{24}O_3$ | 99.73% | 14.71 | $CH_3CN/H_2O$ = 62/38<br>Flow rate: 0.8 mL/min<br>SinoChrom ODS-BP 5μm (30℃) |
| Celastrol | $C_{29}H_{38}O_4$ | 99.80% | 12.91 | CH3OH / CH3COOH = 90/10 20min, 90/10-100/0,30min<br>Flow rate: 0.8 mL/min<br>SinoChrom ODS-BP 5μm (40℃) |
| Triptolide | $C_{20}H_{24}O_6$ | 99.78% | 17.72 | $CH_3CN/H_2O$ = 30/70<br>Flow rate: 1.0 mL/min<br>SinoChrom ODS-BP 5μm (40℃) |
| Wilforlide A | $C_{30}H_{46}O_3$ | 98.07% | 18.50 | $CH_3CN/H_2O$ = 85/15<br>Flow rate: 1.0 mL/min<br>SinoChrom ODS-BP 5μm (70℃) |
| Triptonide | $C_{20}H_{22}O_6$ | 98.67% | 15.89 | $CH_3CN/H_2O$ = 42/58<br>Flow rate: 1.0 mL/min<br>SinoChrom ODS-BP 5μm (40℃) |
| Demethylzeylasteral | $C_{29}H_{36}O_6$ | 99.63 | 12.00 | $CH_3CN/H_2O$ = 70/30<br>Flow rate: 1.0 mL/min<br>SinoChrom ODS-BP 5μm (35℃) |

**The NMR and MS spectroscopic data of compounds**

All the $^{1}H$ NMR spectra of compounds were recorded on an Avance Bruker NMR spectrometer operating at 500 MHz on proton. Mass measurements were performed on a LC-MSD-TOF instrument from Agilent technologies in positive electrospray mode. Samples were automatically injected using a solvent containing 50 % acetonitrile and 50 % a solution of formic acid ( 0.1 % ) in water at a flow rate of 0.5 mL/min. The electrospray voltage was set at 4000 V, the fragmentor at 200 V and the source temperature at 350 ℃. Either protonated molecular ions $[M + H]^{+}$ or sodium adducts $[M + Na]^{+}$ were detected in the mass spectra except triptolide. For triptolide, the $[M -H]^{-}$ was detected in the mass spectra.

**Triptophenolide** White powder, $^{1}H$ NMR (500 MHz, CDCl3): 7.14 (1H, d), 6.96 (1H, d), 4.73-4.77 (2H, m), 1.96-2.26 (5H, m), 1.54-1.55 (1H, m), 1.19-1.25 (1H, m), 1.12 (3H, s), 1.02 (3H, d, J = 5.0 Hz), 0.87 (3H, d, J = 5.0 Hz). TOF MS (ESI), m/z $[M + H]^{+}$: Calc'd, 313.180, found, 313.31.

**Demethylzeylasteral** Yellow powder, $^{1}H$ NMR (500 MHz, CDCl3): δ10.36 (1H, s), 7.34 (1H, s), 6.58 (1H, s), 7.04 (1H, d), 1.76-2.02 (2H, m), 1.74 (3H, s), 1.33 (3H, s), 1.30 (3H, s), 1.31-1.72 (13H, m), 1.10 (6H, s), 1.04 (3H, s), 1.03 (3H, s). TOF MS (ESI), m/z $[M + H]^{+}$: Calc'd, 481.259, found, 481.37.

**Triptonide** White powder, $^{1}H$ NMR (500 MHz, CDCl3): δ4.67-4.69 (2H, m), 3.90 (1H, d), 3.37-3.52 (3H, m), 3.15-3.18 (1H, m), 2.66-2.94 (3H, m), 2.26-2.54 (3H, m), 1.58-2.03 (3H, m), 1.25 (6H, s), 1.02 (3H, s). TOF MS (ESI), m/z $[M + H]^{+}$: Calc'd, 359.149, found, 359.20.

**Celastrol** Red powder, $^{1}H$ NMR (500 MHz, CDCl3): δ7.05 (1H, d), 6.49 (1H, s), 6.31 (1H, d), 5.30 (1H, s), 2.48 (1H, d), 2.21 (3H, s), 2.10-2.17 (4H, m), 1.43-1.77 (11H, m), 1.44 (3H, s), 1.42 (3H, s), 1.34 (3H, s), 1.28 (3H, s), 1.09 (1H, d)，0.55 (3H, s). TOF MS (ESI), m/z $[M + H]^{+}$: Calc'd, 451.285., found 451.22.

**Wilforlide A** White powder, $^{1}H$ NMR (500 MHz, CDCl3): δ5.29 (1H, m), 4.15 (1H, d), 3.21 (1H, m), 2.24(1H, d), 2.10-2.16 (1H, m), 1.86-1.89 (5H, m), 1.37-1.62 (12H, m), 1.21 (3H, s), 1.08-1.16 (2H, m), 1.07 (3H, s), 0.99 (3H, s), 0.95 (3H, s), 0.94 (3H, s), 0.93 (3H, s), 0.87 (3H, s), 0.79(3H, s), 0.74(1H, d). TOF MS (ESI), m/z $[M + Na]^{+}$: Calc'd, 477.334, found, 477.32.

**Triptolide** White powder, $^{1}H$ NMR (500 MHz, CDCl3): δ4.68-4.69 (2H, m), 3.90 (1H, d), 3,51(1H, d), 3.40 (1H, d), 3.37 (1H, d), 2.73(1H, d), 2.68-2.70 (1, m), 1.96-2.26 (5H, m), 1.54-1.55 (1H, m), 1.19-1.25 (1H, m), 1.12 (3H, s), 1.02 (3H, d), 0.87 (3H, d). TOF MS (ESI), m/z $[M - H]^{-}$: Calc'd, 359.149, found,

359.30.

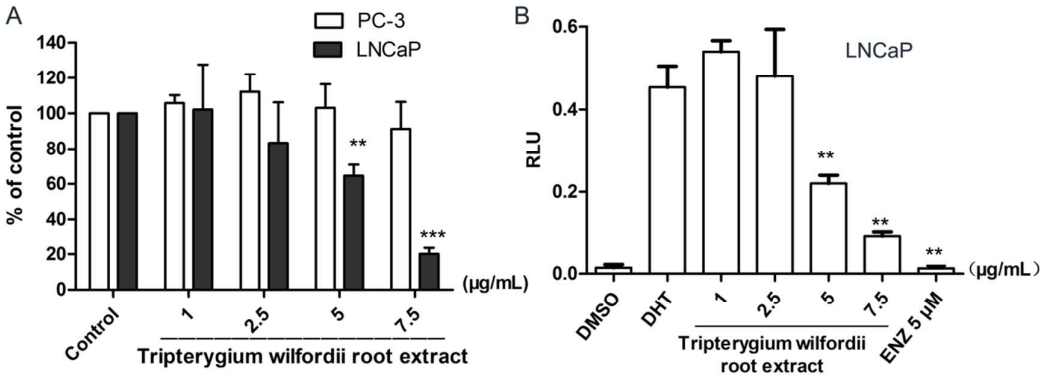


**Figure S1.** A) Growth inhibitory effects of *Tripterygium wilfordii* root extract in the PC-3 and LNCaP cells; B) Effect of Tripterygium *wilfordii* root extract on AR activity in LNCaP cells. Experiments were in triplicate. All results are shown as mean ± s.d. *P < 0.05, **P < 0.01 vs DMSO group. ENZ: enzalutamide; DHT: dihydrotestosterone.

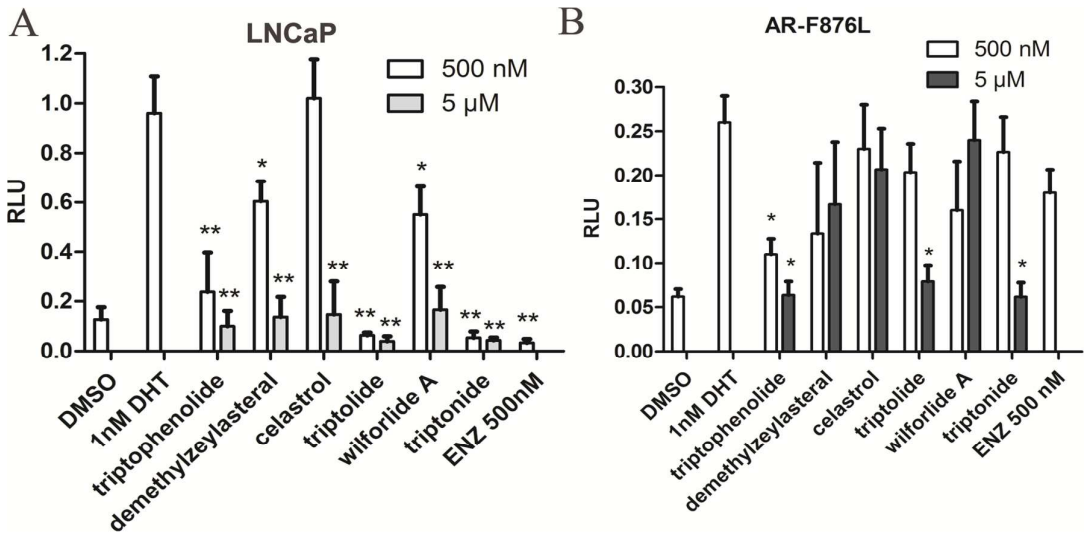


**Figure S2.** A) Effect of *Tripterygium wilfordii* components on AR activity in LNCaP cells; B) Effect of *Tripterygium wilfordii* components on F876L AR activity in PC-3 cells, Plasmids F876L ARs are transiently transfected in PC-3 cells. Experiments were in triplicate. All results are shown as mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001 vs DMSO group. ENZ: enzalutamide; DHT: dihydrotestosterone.

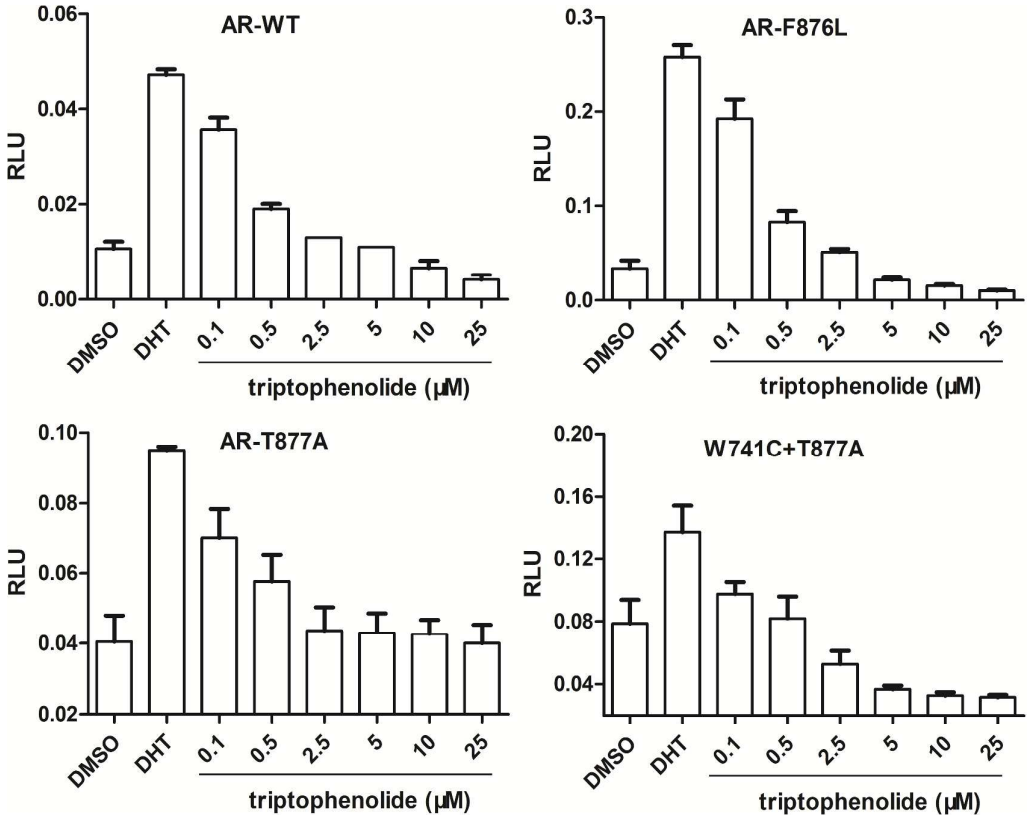


**Figure S3.** The results of the luciferase assay of the ARs activity (induced by 1nM DHT) for triptophenolide within the 0.1-25 μM concentration range. Experiments were in triplicate. All results are shown as mean ± s.d.

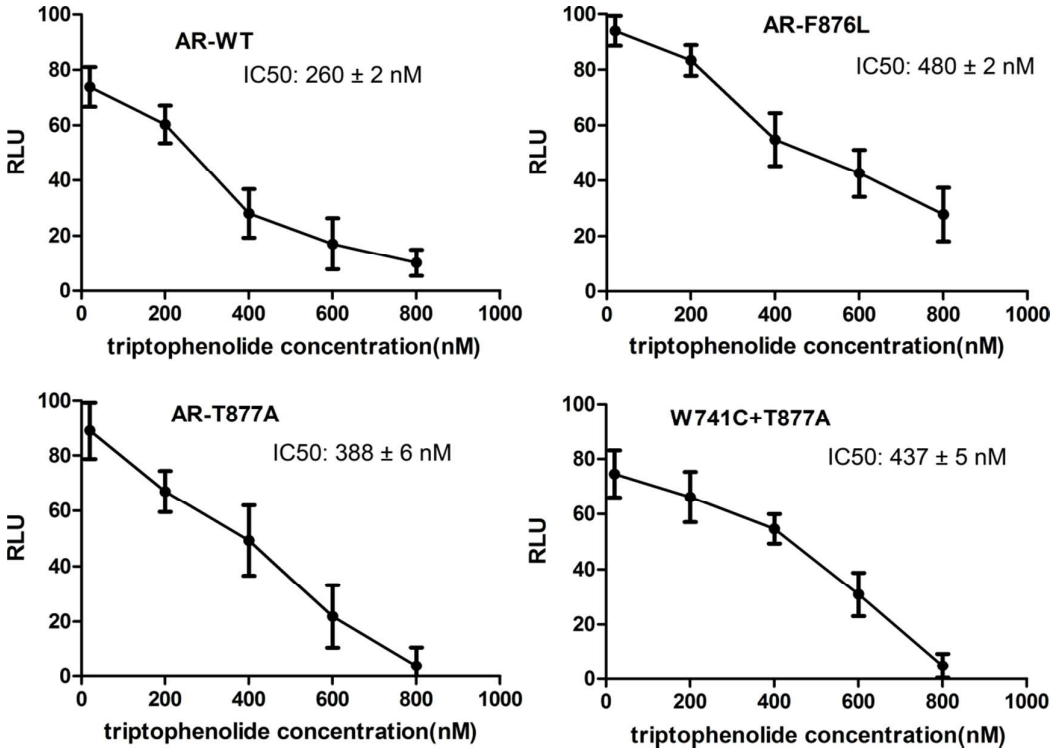


**Figure S4.** The IC50 values of triptophenolide on wild type and mutant ARs' activity. Experiments were in triplicate. All results are shown as mean ± s.d.

**Table S3.** The measured value of RT-PCR assay.

| ( nM ) | PSA | | GAPDH | | 2△Ct | -2△△Ct | |
|---|---|---|---|---|---|---|---|
| | 20.00 | | 18.49 | | | | $2^{-\triangle\triangle Ct}$ |
| DHT 1 | 19.38 | 39.38 | 18.38 | 36.87 | 2.51 | | |
| | 18.30 | | 16.84 | | | | |
| TRI 50 | | 18.30 | | 16.84 | 2.92 | -0.41 | 0.8675 |
| | 18.14 | | 16.06 | | | | |
| TRI 500 | 18.22 | 36.36 | 15.95 | 32.01 | 4.35 | -1.84 | 0.5285 |
| | 19.40 | | 15.86 | | | | |
| TRI 5000 | 20.03 | 39.43 | 16.00 | 31.86 | 7.57 | -5.06 | 0.1731 |
| | 22.43 | | 19.04 | | | | |
| ENZ 500 | 22.08 | 44.51 | 19.12 | 38.16 | 6.35 | -3.84 | 0.2643 |
| | | | | | | | |
| ( nM ) | PSA | | GAPDH | | 2△Ct | -2△△Ct | |
| | 18.26 | | 15.81 | | | | $2^{-\triangle\triangle Ct}$ |
| DHT 1 | 17.95 | 36.21 | 16.06 | 31.87 | 4.34 | | |
| | 16.81 | | 14.70 | | | | |
| TRI 50 | 17.45 | 34.26 | 15.05 | 29.75 | 4.51 | -0.17 | 0.9428 |
| | 16.17 | | 13.23 | | | | |
| TRI 500 | 17.12 | 33.29 | 13.80 | 27.03 | 6.26 | -1.92 | 0.5141 |
| | 17.26 | | 13.09 | | | | |
| TRI 5000 | 18.50 | 35.76 | 12.38 | 25.47 | 10.29 | -5.95 | 0.1272 |
| | 18.81 | | 15.83 | | | | |
| ENZ 500 | 19.67 | 38.48 | 15.64 | 31.47 | 7.01 | -2.67 | 0.3964 |

Note: ENZ, enzalutamide; DHT, dihydrotestosterone; TRI, triptophenolide.

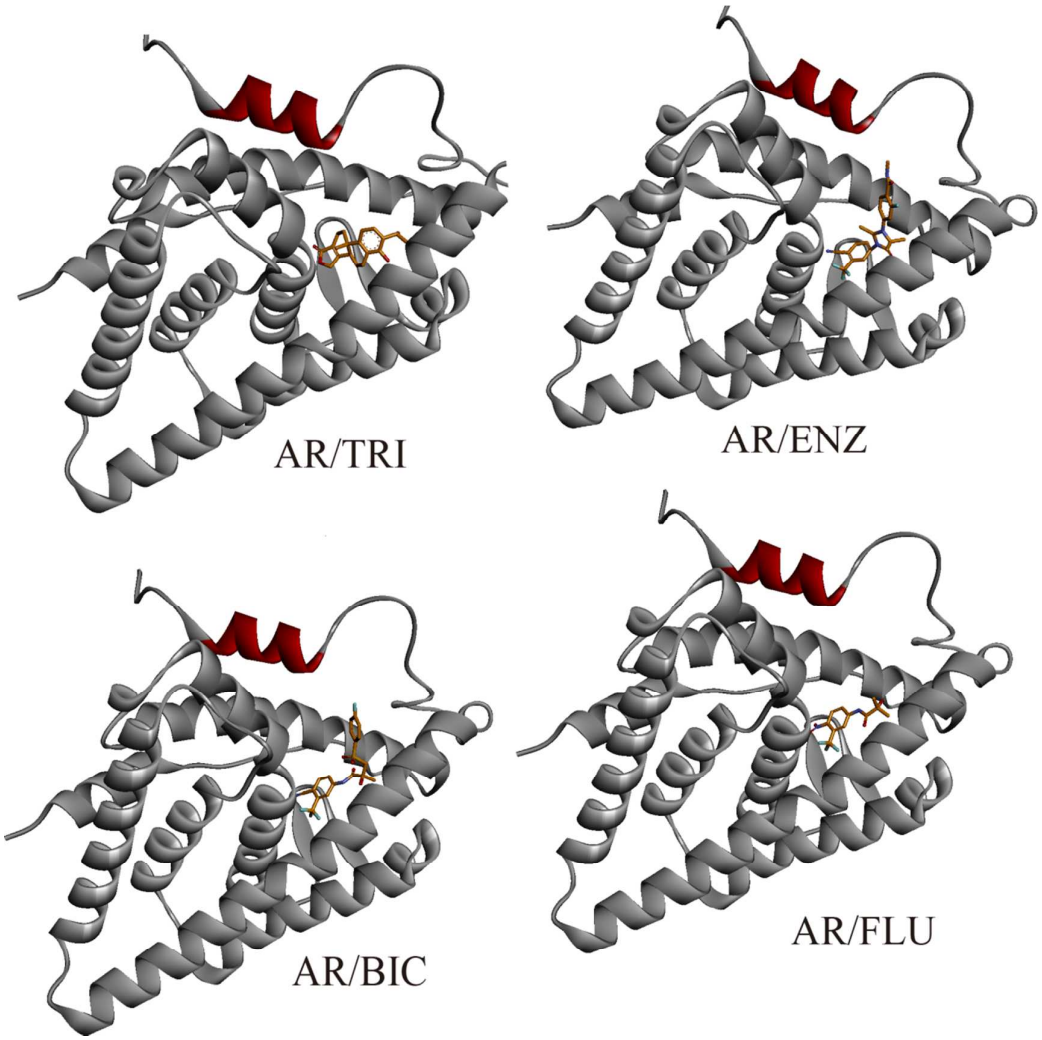


**Figure S5.** The predicted binding modes for triptophenolide, as well as three other anti-androgens (Enzalutamide, Bicalutamide, Hydroxyflutamide) in the HBP of AR. The proteins are in ribbon, and the compounds are in stick, carbon in orange.

**Table S4.** The ranking of docking score for TRI and other clinical used antiandrogens.

| Compounds | Affinity (By our assay )[a] | Affinity (Reported) | Docking Score (London dG in MOE) |
|---|---|---|---|
| Enzalutamide (ENZ) | 294 nM | 21.4 nM [b 1] | -12.51 kcal/mol |
| Triptophenolide (TRI) | 467 nM | / | -11.80 kcal/mol |
| Bicalutamide (BIC) | ND | ~400 nM[c 2] | -11.14 kcal/mol |
| Hydroxyflutamide (FLU) | ND | 900 nM[c] | -10.92 kcal/mol |

a. The AR fluorescence polarization (FP) assay for competitive binding with androgen in the HBP was performed with PolarScreen AR competitor assay kit (P3018, Invitrogen);
b. IC50 values calculated as the concentration of drug required to produce 50% displacement of $^{18}$F-FDHT from androgen receptors.
c. IC50 values calculated as the concentration of drug required to produce 50% displacement of [3H]5a-DHT from androgen receptors.

1. Tran, C.; Ouk, S.; Clegg, N. J.; Chen, Y.; Watson, P. A.; Arora, V.; Wongvipat, J.; Smith-Jones, P. M.; Yoo, D.; Kwon, A.; Wasielewska, T.; Welsbie, D.; Chen, C. D.; Higano, C. S.; Beer, T. M.; Hung, D. T.; Scher, H. I.; Jung, M. E.; Sawyers, C. L., Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* **2009,** *324* (5928), 787-90.

2. Kolvenbag, G. J.; Furr, B. J.; Blackledge, G. R., Receptor affinity and potency of non-steroidal antiandrogens: translation of preclinical findings into clinical activity. *Prostate Cancer Prostatic Dis* **1998,** *1* (6), 307-314.

| AR-WT | +DMSO | | | | | +DHT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | 50nM | 500nM | 5μM | ENZ 500nM | DHT 1nM | 50nM | 500nM | 5 μM | ENZ 500nM |
| Firefly | 437806 | 234496 | 195606 | 137058 | 153577 | 1268414 | 467924 | 330115 | 304562 | 85646 |
| | 423953 | 181493 | 192320 | 160998 | 188547 | 1373083 | 606851 | 366412 | 189749 | 246161 |
| | 370048 | 209273 | 199990 | 145512 | 184059 | 1053204 | 606200 | 280564 | 208620 | 213213 |
| Renilla | 15786654 | 11737190 | 11525739 | 13816524 | 13995126 | 21541002 | 15936007 | 14872618 | 17326644 | 12529020 |
| | 15931298 | 13170927 | 11287618 | 15339563 | 13290746 | 21920002 | 17409582 | 14239389 | 14663011 | 14943361 |
| | 18415800 | 16005056 | 18445982 | 17608218 | 16295326 | 22152850 | 16632897 | 14409273 | 21379646 | 19334408 |
| Ratio | 0.028 | 0.020 | 0.017 | 0.010 | 0.011 | 0.059 | 0.029 | 0.022 | 0.018 | 0.007 |
| | 0.027 | 0.014 | 0.017 | 0.010 | 0.014 | 0.063 | 0.035 | 0.026 | 0.013 | 0.016 |
| | 0.020 | 0.013 | 0.011 | 0.008 | 0.011 | 0.048 | 0.036 | 0.019 | 0.010 | 0.011 |

| AR-F876L | +DMSO | | | | | +DHT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | 50nM | 500nM | 5μM | ENZ 500nM | DHT 1nM | 50nM | 500nM | 5 μM | ENZ 500nM |
| Firefly | 94807 | 73369 | 70383 | 26467 | 200752 | 316179 | 166117 | 95698 | 76230 | 231012 |
| | 33997 | 79790 | 63478 | 34373 | 258359 | 414901 | 82726 | 77397 | 55361 | 470195 |
| | | 49991 | 27249 | 39650 | 182548 | 360672 | | | 117374 | |
| Renilla | 23895368 | 23929450 | 24898810 | 20415726 | 24954884 | 24707778 | 16066675 | 17685344 | 27684260 | 26682684 |
| | 20767712 | 34403048 | 28707996 | 25449322 | 27845868 | 29025548 | 12007366 | 16277931 | 28295732 | 28244670 |
| | | 25738538 | 14456291 | 29747364 | 14070836 | 24716170 | | | 41772876 | |
| Ratio | 0.004 | 0.003 | 0.003 | 0.001 | 0.008 | 0.013 | 0.01 | 0.005 | 0.003 | 0.009 |
| | 0.002 | 0.002 | 0.002 | 0.001 | 0.009 | 0.014 | 0.007 | 0.005 | 0.002 | 0.017 |
| | | 0.002 | 0.002 | 0.001 | 0.013 | 0.015 | | | 0.003 | |

| AR-T877A | +DMSO | | | | | | +DHT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | 50nM | 500nM | 5μM | ENZ 500nM | FLU 5μM | DHT 1nM | 50nM | 500nM | 5 μM | ENZ 500nM | FLU 5μM |
| Firefly | 428952 | 322032 | 277375 | 275211 | 344769 | 814400 | 1028502 | 655795 | 419989 | 409347 | 240729 | 722555 |
| | 360761 | 334240 | 239487 | 270561 | 246700 | 831606 | 901511 | 519040 | 478534 | 424721 | 260446 | 685107 |
| | 410678 | 272680 | 255339 | 261025 | 281051 | 657728 | 970449 | 606103 | 421398 | 538090 | 225613 | 817679 |
| Renilla | 12255771 | 10734400 | 10273148 | 11008440 | 12769222 | 12926976 | 13215038 | 9398219 | 9604481 | 9243665 | 8560810 | 11608609 |
| | 12025367 | 12379259 | 9579480 | 11763500 | 12335000 | 14589570 | 13019214 | 10262667 | 10029756 | 9356209 | 8494739 | 12062849 |
| | 12444788 | 9402759 | 9820731 | 10876021 | 11710458 | 10962133 | 12426441 | 10621903 | 9979706 | 11592867 | 8653479 | 12948827 |
| Ratio | 0.035 | 0.030 | 0.027 | 0.025 | 0.027 | 0.063 | 0.078 | 0.07 | 0.044 | 0.044 | 0.028 | 0.062 |
| | 0.030 | 0.027 | 0.025 | 0.023 | 0.020 | 0.057 | 0.069 | 0.051 | 0.048 | 0.045 | 0.031 | 0.057 |
| | 0.033 | 0.029 | 0.026 | 0.024 | 0.024 | 0.060 | 0.078 | 0.057 | 0.042 | 0.046 | 0.026 | 0.063 |

| W741C+T877A | +DMSO | | | | | | | +DHT | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | 50nM | 500nM | 5μM | ENZ 500nM | FLU 5μM | BIC 5μM | DHT 1nM | 50nM | 500nM | 5 μM | ENZ 500nM | FLU 5μM | BIC 5μM |
| Firefly | 2138134 | 2817716 | 2310359 | 2112355 | 1658694 | 4000300 | 5812393 | 4355287 | 3575146 | 2915319 | 1904401 | 2955609 | 4451263 | 7560061 |
| | 3078956 | 3302883 | 2789832 | 2800530 | 2695231 | 6370721 | 6282225 | 4379252 | 4039858 | 2652780 | 3343571 | 2607253 | 4193363 | 5841136 |
| | 2647249 | 3699849 | 2523806 | 3183301 | 2231790 | 5565393 | 5669328 | 4454328 | 3968949 | 3070609 | 2473140 | 2962324 | 4749484 | 4443835 |
| Renilla | 7702930 | 7788668 | 10331952 | 10984217 | 10398809 | 10030433 | 14189078 | 33313648 | 34918900 | 37130400 | 39156912 | 48642316 | 37374828 | 40737272 |
| | 17754168 | 26863862 | 34823180 | 40632980 | 48728460 | 45675928 | 41275800 | 30955056 | 35067296 | 34692196 | 48908448 | 46494284 | 36961584 | 40452848 |
| | 36575908 | 37721212 | 43759448 | 51324768 | 52935792 | 45665016 | 38166624 | 37987812 | 30532860 | 30748022 | 42089316 | 47435712 | 42436236 | 37078712 |
| Ratio | 0.083 | 0.007 | 0.069 | 0.065 | 0.048 | 0.115 | 0.136 | 0.131 | 0.123 | 0.079 | 0.049 | 0.061 | 0.119 | 0.132 |
| | 0.078 | 0.082 | 0.080 | 0.069 | 0.055 | 0.109 | 0.132 | 0.141 | 0.115 | 0.076 | 0.068 | 0.056 | 0.113 | 0.144 |
| | 0.072 | 0.076 | 0.058 | 0.062 | 0.042 | 0.122 | 0.140 | 0.136 | 0.130 | 0.100 | 0.059 | 0.062 | 0.112 | 0.120 |

**Figure S6.** The measured value of dual-luciferase assay with triptophenolide treated. ENZ, enzalutamide; DHT, dihydrotestosterone; BIC, bicalutamide; FLU, flutamide.