Supplementary Materials and Methods

Characterization of a new class of androgen receptor antagonists with potential therapeutic application in advanced prostate cancer

Huifang Li,†1 Mohamed DH Hassona,†1,5 Nathan A. Lack,†1,2 Peter Axerio-Cilies,1 Eric Leblanc,1 Natalia Kanaan,1 Kate Frewin,1 Kriti Singh,1 Hans Adomat,1 Konrad J. Böhm,3 Helge Prinz,4 Emma Tomlinson Guns,§1 Paul S. Rennie,§1 and Artem Cherkasov§*1

1Vancouver Prostate Centre, University of British Columbia, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6, Canada
2School of Medicine, Koç University, Istanbul, 34450, Turkey
3Leibniz Institute for Age Research (FLI), Beutenbergstrasse 11, D-07745 Jena, Germany
4Institute of Pharmaceutical and Medicinal Chemistry, Westfälische Wilhelms-Universität, Corrensstrasse 48, D-48149 Münster, Germany
5Faculty of Pharmacy, Helwan University, Egypt
† Authors contributed equally to this work
§ Dr. Cherkasov’s, Dr. Guns’ and Dr. Rennie’s labs made equal contributions to this work.
*Corresponding author: Artem Cherkasov, Vancouver Prostate Centre, University of British Columbia, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6, Canada. Phone: 604-875-4111. Fax: 604-875-5654. E-mail:artc@interchange.ubc.ca
Materials and Methods

Structure-based Virtual screening (SBVS)

*Ligand preparation:* Compounds were constructed using the Builder module in Molecular Operating Environment (MOE) version 2010 (1). Hydrogen atoms were added, and energy minimization was performed with the MMFF94x force field, and then optimized structures were exported into the Maestro suite (2) in sdf file format.

*Protein preparation:* The crystal structure of AR was prepared in the protein preparation wizard module in Maestro. All solvent molecules were deleted and the bond order for the ligand and protein were adjusted. The missing hydrogen atoms were added, and side chains were energy-minimized using the OPLS-2005 force field, as implemented by Maestro. The crystallographic ligand was used to define the ligand binding region for both glide and eHiTs docking programs with default settings.

Molecular Dynamics (MD)

The initial coordinates for this study were taken from the docked poses of VPC-3022 and human AR (2PNU.pdb) (3). In order to adjust the ionization state of all the titratable residues in the protein, the empirical propKa program (4) was used. It should be pointed out that all the residues were found at their standard protonation state at pH 7. The missing residues in the 2PNU.pdb (from 845 to 849) were added using 3L3X.pdb (5) as a template within MOE software (1). The next step was to add hydrogens to the structure and apply several optimization algorithms. After that, five counterions were added in order to maintain the charge of the system neutral. The optimized protein was placed in an orthorhombic box of pre-equilibrated water molecules (100 X 80 X 80 Å³) and all the water molecules with its oxygen atom closer than 2.8 Å from any heavy atom of the system were deleted. The box of water molecules was then
relaxed and, finally, the whole system. Cutoffs to treat the non-bonding interactions were applied using a fswitch scheme, with a cutoff distance in the range of 14 to 16 Å. Once the system was relaxed, a 10 ns long MD at 310 K was carried out by means of NAMD v.2.6 program (6), using the CHARMM 22 force field (7-9).

Chemistry

Melting points were determined with a Kofler melting point apparatus and are uncorrected. Spectra were obtained as follows: $^1$H NMR spectra were recorded with Varian Gemini 200 (50, 3 MHz) or Varian Mercury 400 plus (400 MHz), respectively. NMR signals were referenced to TMS ($\delta = 0$ ppm) or solvent signals and recalculated relative to TMS. Fourier-transform IR spectra were recorded on a Bio-Rad laboratories Typ FTS 135 spectrometer and analysis was performed with WIN-IR Foundation software. Mass spectra were obtained on Finnigan GCQ and LCQ apparatuses applying electron beam ionization (EI).

The purity of all target compounds was determined by elemental analyses. Elemental analyses were performed at the Münster microanalysis laboratory, using a Vario EL elemental analyzer from Elementar Analysensysteme GmbH Hanau, and all values were within ± 0.4% of the calculated composition.

All organic solvents were appropriately dried or purified prior to use. Aryl aldehydes and 10$H$-anthracen-9-one were obtained from commercial sources and used without further purification. Purification by chromatography refers to column chromatography on silica gel (Macherey-Nagel, 70-230 mesh). In most cases, the concentrated pure fractions obtained by chromatography using the indicated eluants were treated with a small amount of hexane to induce precipitation.

All newly synthesised compounds displayed $^1$H NMR and MS spectra consistent with the
assigned structure. Yields have not been optimized. Analytical TLC was done on Merck silica 60 F254 alumina coated plates (E. Merck, Darmstadt). (See details on the preparation of 10-Benzylidene-10H-anthracen-9-ones in Supplementary Materials and Methods)

**Preparation of 10-Benzylidene-10H-anthracen-9-ones**

10-(2,6-dimethoxybenzylidene)-10H-anthracen-9-one (VPC-3034).

10H-Anthracen-9-one (0.97 g, 5 mmol) and 2,5-dimethoxybenzaldehyde (0.83 g, 5 mmol) were suspended in 30 mL of dry pyridine under nitrogen. Piperidine (5 mL) was added and the mixture was thereafter heated on an oil bath (130 °C) under nitrogen until the reaction was complete (TLC control). Then, the reaction mixture was cooled to room temperature, poured on water (300 mL) and acidified with 6N HCl. The precipitate was extracted with CH2Cl2 (3 × 50 mL), the organic phase was dried over Na2SO4 and then concentrated in vacuo. Purification by silica gel chromatography (CH2Cl2) afforded VPC-3034 as a fine yellow powder (0.11 g, 6% yield): mp 147-148 °C; FTIR 1655 cm⁻¹; ¹H NMR (CDCl3, 400 MHz) δ 8.30-8.24 (m, 2H), 8.10-8.08 (m, 1H), 7.67-7.59 (m, 2H) and 7.57 (s, 1H), 7.51-7.47 (m, 1H), 7.30-7.25 (m, 1H), 6.89-6.82 (m, 2H), 6.65 (d, 2H), 3.84 (s, 3H), 3.54 (s, 3H); MS (EI, 70 eV) m/z (%) 342 (100); Anal. (C23H18O3, 342.13) calcd C 80.68, H 5.30; found C 80.69, H 5.42.

10-(3-Hydroxybenzylidene)-10H-anthracen-9-one (VPC-3033).

This was prepared from 10H-anthracen-9-one (0.97 g, 5 mmol) and 3-hydroxybenzaldehyde (0.61 g, 5 mmol) in a similar manner as described for VPC-3034. Purification by silica gel chromatography (CH2Cl2) afforded VPC-3033 as a fine yellow powder (0.26 g, 17% yield): mp 183-184 °C; FTIR 3294, 1643 cm⁻¹; ¹H NMR (CDCl3, 400 MHz) δ 8.28-8.25 (m, 1H), 8.20-8.18 (m, 1H), 7.99 (dd, 1H, J = 8.01 Hz, J = 0.59 Hz), 7.66-7.62 (m, 1H), 7.56-7.48 (m, 3H), 7.40-7.36 (m, 1H), 7.27-7.20 (m, 2H), 6.89 (dd, 1H, J = 7.62 Hz, J = 0.78 Hz).
Hz), 6.82-6.80 (m, 2H), 5.48 (s, 1H); MS (EI, 70 eV) m/z (%) 299 (19), 298 (91), 297 (100); Anal. (C_{21}H_{14}O_{2}, 298.10) calcd C 84.54, H 4.73; found C 84.89, H 4.54.

10-(2-Thienylmethylene)-10H-anthracen-9-one (VPC-3026).

This was prepared from 10H-anthracen-9-one (1 mmol, 0.19 g) and thiophene-2-carbaldehyde (0.13 g, 1 mmol) in a similar manner as described for VPC-3034. Purification by silica gel chromatography (ethyl acetate/hexane, 1:1) afforded VPC-3026 as a fine yellow powder (0.18 g, 63% yield): mp 164-166 °C (lit. (10) 170 °C); FTIR 1659 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 200 MHz) \(\delta\) 8.28-8.24 (m, 2H), 8.14-8.09 (m, 1H), 7.96 (d, 1H, \(J = 8.01\) Hz), 7.67-7.59 (m, 1H), 7.52-7.57 (m, 4H), 7.33-7.30 (m, 1H), 7.19-7.17 (m, 1H), 7.01-6.96 (m, 1H); MS (EI, 70 eV) m/z (%) 288 (70), 287 (100); Anal. (C\(_{19}\)H\(_{12}\)OS, 288.06) calcd C 79.14, H 4.19; found C 78.97, H 4.35.

10-(2, 3-Dimethoxybenzylidene)-10H-anthracen-9-one (VPC-3028).

The title compound was prepared from 10H-anthracen-9-one (5 mmol, 0.97 g) and 2, 3-dimethoxybenzaldehyde (0.83 g, 5 mmol) in a similar manner as described for VPC-3034. Purification by silica gel chromatography (CH\(_2\)Cl\(_2\)) afforded VPC-3028 as yellow needles (0.21 g, 12% yield): mp 130-131 °C; FTIR 1659 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 8.29 (dd, 1H, \(J = 7.8\) Hz, \(J = 1.4\) Hz), 8.25 (dd, 1H, \(J = 7.8\) Hz, \(J = 1.4\) Hz), 8.09 (d, 1H, \(J = 8.0\) Hz), 7.70-7.63 (m, 2H), 7.58-7.48 (m, 2H), 7.41 (td, 1H, \(J = 7.7\) Hz, \(J = 1.1\) Hz), 7.29-7.22 (m, 2H), 6.90-6.85 (m, 2H), 6.69-6.63 (m, 1H); MS (EI, 70 eV) m/z 342 (100); Anal. (C\(_{23}\)H\(_{18}\)O\(_3\), 342.13) calcd C 80.68, H 5.30; found C 80.38, H 5.58.

All other compounds were synthesized as previously described (11).
References for the Supplementary Materials and Methods


Supplementary Figures and Tables

Figure S1. Chemical structures of current antiandrogens with common motifs highlighted.

Figure S2. Inhibition of VPC-3022 against wild-type AR in HeLa-AR cells. HeLa-AR cells transfected with ARR3tk-luciferase reporter were treated with VPC-3022 for 24 hours in various concentrations in the presence of 0.1 nM R1881. Error bars indicate standard deviation.

Figure S3. AR mRNA levels are unaffected upon treatment with VPC-3022 and VPC-3033. Total RNA was extracted after treatment of LNCaP and MDV3100-resistant cells with VPC-3022 and VPC-3033 for 24 hours at the indicated concentrations in the presence of 0.1 nM R1881. Quantitative RT-PCR analysis of AR expression was performed and normalized to 18S rRNA levels. Error bars indicate standard deviation.

Figure S4. (A) Effect of VPC-3022 on AR activity in HeLa-AR cells in the presence of cyclohexamide. HeLa-AR cells were treated with serially-diluted concentrations of VPC-3022 (0 – 100 µM) with or without 10 µM of cyclohexamide for 8 hours, and then lysed with passive lysis buffer. (B) AR degradation caused by VPC-3031 and VPC-3041 in HeLa-AR cells. HeLa-AR cells were treated by VPC-3031 and VPC-3041 at different concentrations in the presence of 0.1 nM R1881.

Figure S5. The binding pose of DHT (A) and VPC-3022 with AR by molecular docking (B) and binding pose of VPC-3022 with AR after 10 ns molecular dynamics (C); RMSD values of the protein backbone, protein-ligand, ligand in the 10 ns molecular dynamics for AR-DHT (D) and AR-VPC-3022 (E).

Figure S6. (A) Inhibition of in vitro polymerization of tubulin at 37 °C by various concentrations of VPC-3033; turbidity was recorded at 360 nM. The steady-state tubulin assembly level in the
absence of inhibitor was set at 100%. (B) IC\textsubscript{50} values were determined by sigmoidal fitting of the plot of the steady state levels of tubulin assembly (taken after 45 min) against drug concentration and represent the concentration for 50% inhibition of the maximum tubulin polymerization level.