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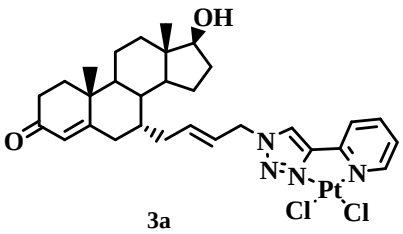
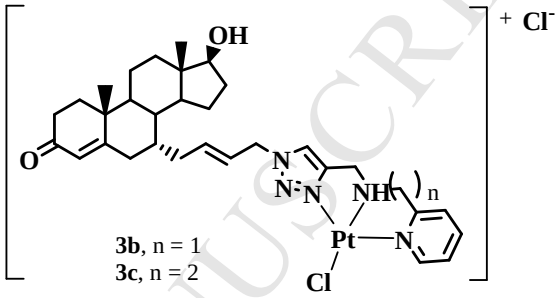
Graphical abstract

Two types of testosterone-platinum(II) complexes (compounds **3a** and **3b,c**) were made and tested for their antiproliferative activity. Hybrid **3a** was as active as cisplatin and showed selectivity on androgen-dependent prostate cancer cells LNCaP.



3a



3b, n = 1
3c, n = 2

Second-generation testosterone-platinum(II) hybrids for site-specific treatment of androgen receptor positive prostate cancer: Design, synthesis and antiproliferative activity

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Abbreviation list: T, testosterone; T-Pt(II), testosterone-platinum(II) hybrid; PCa, prostate cancer; AR, androgen receptor.

Abstract

Prostate cancer is the most diagnosed type of cancer in men in Canada. One out of eight men will be stricken with this disease during the course of his life. It is noteworthy that, at initial diagnoses 80-90% of cancers are androgen dependent. Hence, the androgen receptor is a viable biological target to be considered for drug targeting. We have developed a new generation of testosterone-Pt(II) hybrids for site-specific treatment of hormone-dependent prostate cancer. The hybrid molecules are made from testosterone using an eight-step reaction sequence with about 7% overall yield. They are linked with a stronger tether chain between the testosterone moiety and the Pt(II) moiety in comparison to our first generation hybrids. The new hybrids were tested on hormone-dependent and –independent prostate cancer cell lines. The hybrid **3a** presents the best antiproliferative activity and was selective on hormone-dependent prostate cancer with IC_{50} of 2.2 μM on LNCaP (AR+) in comparison to 13.3 μM on PC3 (AR-) and 8.8 μM on DU145 (AR-) prostate cancer cells. On the same cell lines, CDDP displayed IC_{50} of 2.1 μM, 0.5 μM and 1.0 μM, respectively. Remarkably, hybrid **3a** was inactive on both colon carcinoma (HT-29) and normal human adult keratinocyte cells (HaCat) with an IC_{50} of > 25 μM. This is not the case for CDDP showing IC_{50} of 1.3 μM and 5.1 μM on HT-29 and HaCat cells, respectively. The potential for selective activity on androgen-receptor positive prostate cancer cells is confirmed with hybrid **3a** giving new hope for an efficient and less toxic platinum-based treatment of prostate cancer patients.

Keywords. testosterone; prostate cancer; SRB assay; hybrid anticancer agent; drug-targeting.

1. Introduction

Cancer is a difficult disease to cure if not detected and treated at its early stages. It is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018 [1]. Worldwide, about 1 in 6 deaths is due to cancer. Prostate cancer (PCa) is a major health problem afflicting men with an estimate of 1.28 million cases worldwide [1]. In fact, PCa is the fourth most frequent cancer in the world with 7.1% of all cases, after lung (11.6%), breast (11.6%), and colorectal (10.2%) cancers. In Canada, PCa is estimated to be ranked first among all cancer cases diagnosed in the Canadian men with about 21,300 new cases [2]. The projected mortality for PCa is about 4,100 cases and is the third most lethal cancer in men [2]. Most PCa (74%) are diagnosed at stage I or II and are generally hormone-dependent [2,3]. Considering the latter fact, it is theoretically possible to use the androgen receptor (AR) as a biological target to direct an anticancer drug to PCa cells.

Today, despite the use of innovative anticancer drugs there is still a need to improve selectivity, efficacy and, at the same time to reduce the many incapacitating side effects of cancer treatments. Hence, there is great scientific interest in using hybrid molecules to combat several types of diseases, including cancer. One can appreciate the power of hybridization technology used during many drug discovery projects through recent reviews on the subject [4-9].

We have investigated the use of testosterone as a carrier molecule united to different DNA alkylating agents [10,11]. Two types of testosterone-anticancer agent hybrids were made using either a nitrogen-mustard or a platinum(II) complexes. For example, the testosterone-

chlorambucil hybrid **1** was made and tested in our laboratory with the goal of expanding the use of chlorambucil to PCa [10]. Indeed, chlroambucil (Leukeran®) is a long-standing anticancer drug that is still in use for the treatment certain cancers such as leukemias and lymphomas. The hybrid **1** was as active as chlorambucil and displayed selectivity on hormone-dependent PCa cells [10]. We also developed a first generation of testosterone-platinum(II) hybrid molecules for the treatment of PCa [11]. It was discovered that the thiazolylalanine hybrid **2** possesses the best *in vitro* antiproliferative activity with IC_{50} of 1.8 μM and 1.4 μM on LNCaP (AR+) and PC3 (AR-) cancer cells, respectively. Cisplatin, the reference drug was 3.7 and 5.3 times less potent on the same cell lines [11]. Encouraged by these results, a second-generation testosterone-platinum hybrids **3** was investigated and the results are presented in this manuscript (see Fig. 1). For this second generation of hybrids, it was decided to use a 1,2,3-triazole function (**3**) instead of an ester function (**1** and **2**) to bind the steroid moiety with the platinum(II) moiety in order to obtain chemically stable hybrids for subsequent *in vivo* biological assays. In earlier studies, we investigated using multiple spectroscopic methods the interactions of the hybrid **3a** and its precursor **6a** with several bio-macromolecules (see Scheme 1). The first study showed that both compounds interact with serum proteins (HAS, BSA and β-lactoglobulin) via ionic contacts with BSA forming more stable conjugates than HSA and β-LG [12]. Their interaction with DNA was also studied in our laboratory. Likewise, the drug-DNA conjugation occurs via ionic interactions with **3a** forming more stable DNA adducts than **6a** with $K_{\mathbf{6a}\text{-DNA}}$ =1.80 (±0.5) x $10^5 M^{-1}$ and $K_{\mathbf{3a}\text{-DNA}}$ = 2.3 (±0.8) x $10^5 M^{-1}$ [13]. Finally, it was observed that the interaction with tRNA was also through ionic interactions with **3a** forming more stable tRNA complexes in comparison to **6a**: $K_{\mathbf{3a}\text{-tRNA}}$ = 3.2 (± 0.9) x 10^5 M^{-1} > $K_{\mathbf{6a}\text{-tRNA}}$ = 2.1 (± 0.7) x 10^5 M^{-1} [14]. This manuscript describes

the design, synthesis and characterization of the second-generation testosterone-platinum(II) hybrid. It also presents their antiproliferative activity on several human cancer cell lines.

Insert Fig. 1 and its legend

2. Design and chemistry

Testosterone was selected as the carrier molecule of the hybrids based on its inherent affinity for prostate tissue, to optimize the pharmacokinetics and the pharmacodynamics of testosterone towards AR and to allow the activation of AR signaling pathways. As in our earlier work, the 7α position of testosterone was used as it is nicely located at the midway of the important binding sites (3-C=O and 17-OH groups) involved in essential hydrogen bonds with AR. It is important that these two key groups remain free of steric hindrances to promote strong interactions with AR allowing its activation. As aforementioned, it was decided to construct the new hybrids using a 1,2,3-triazole ring system allowing easy access to the final hybrid molecules via the azide-alkyne cycloaddition coupling reaction [15,16].

The synthetic methodology for the preparation of the second-generation hybrids is described in Scheme 1. Derivative **4** was prepared in good yield via the intermediate 7α-allyl-4-androstene-17β-ol-3-one acetate using a five-step reaction sequence as reported by Bastien *et al.* [10]. Next, a simple substitution of the chloride atom using sodium azide gave derivative **5** with 92% yield. This derivative is now ready for the 1,3-dipolar Huisgen cycloaddition with an appropriate alkyne. So, upon treatment of azide **5** with commercially available 2-ethynylpyridine (**7a**),

sodium carbonate and copper sulfate in a mixture of tetrahydrofuran and water, the 1,2,3-triazole-pyridine ligand **6a** was obtained with 54% yield. The cycloaddition reaction was performed with two additional alkynes, derivatives **7b** and **7c**. These amino-pyridine alkynes were obtained by alkylation of picolylamine and 2-(2-aminoethyl)pyridine with propargyl bromide following a known procedure (Scheme 2) [17]. Compounds **7b** and **7c** were obtained with 50% and 46% yield, respectively. The Huisgen cycloaddition reaction with azide **5** and the relevant alkyne (**7b** and **7c**) gave the 1,2,3-triazole-aminopyridine ligands **6b** and **6c** with 29% and 60% yield, respectively. Finally, complexation of derivatives **6a-c** with potassium tetrachloroplatinate(II) in a mixture of dimethylformamide (DMF) and water led to the testosterone-platinum(II) hybrids (compounds **3a-c**). As expected, the hybrid **3a** (34%) is bound to the bidentate ligand **6a** and the hybrids **3b** (77%) and **3c** (60%) are obtained with tridentate ligands **6b** and **6c**, respectively. All compounds were characterized by their respective infrared (IR), nuclear magnetic resonance spectroscopy (proton and carbon NMR) and by high resolution mass analysis.

Insert scheme 1 and its legend

Insert scheme 2 and tis legend

3. Results and discussion

3.1 Antiproliferative activity on prostate cancer cell lines

The antiproliferative activity of compounds **3a-c** and **6a-c** was assessed using the sulforhodamine B colorimetric test on three PCa cell lines: LNCaP (AR+), PC3 (AR-) and DU145 (AR-) [18,19].

Also, to further verify their biological potential, our products were tested on two additional cell lines: on colon carcinoma (HT-29) and on primary human immortalized keratinocyte cells (HaCat). CDDP was used as a positive control drug in order to better appreciate and compare the biological activity of the synthesized compounds. The results from the antiproliferative activity assay are summarized in Table 1. IC_{50} is the concentration of drug inhibiting cell growth by 50%.

The ligands and platinum complexes were dissolved in DMF at an initial concentration of 25 μM. This concentration was selected as the platinum derivatives **3b** and **3c** were not completely soluble at higher concentrations. The solubility of the platinum complex **3a** was much better. One factor contributing to the solubility of these molecules is their chemical structure that are quite different from one another. Indeed, platinum(II) complex **3a** is obtained with a bidentate ligand while complexes **3b** and **3c** are coordinated with tridentate ligands. The later complexes are charged positively while the complex **3a** is neutral. The literature describes the synthesis estradiol-platinum(II) complexes bonded to a tridentate ligand (2,2':6',2''-terpyridine) displaying similar low solubility [20].

The results show that the ligand precursors **6a-c** are active on all cell tested at the exception of ligand **6c** which is inactive on AR+ PCa cells displaying a IC_{50} > 25 μM. The IC_{50} for the precursor ligands ranged from 6.3 to 20 μM (Table 1). There is no selective activity on any type of cancer cells tested in the panel. This in itself is an interesting result. It is important to note that platinum(II) complex **3a** displays higher activity in comparison to its precursor ligand **6a** on only

two types of cells: LNCaP: 2.2 μM vs 6.3 μM, and DU145: 8.8 μM vs 12.3 μM. Derivative **3a** and **6a** have similar activity on PC3 cells: 13.3 μM vs 11.0 μM. Hence, the precursor ligands are more broadly active than the final platinum complexes. This observation can be explained by the fact that compounds bearing a 1,2,3-triazole ring system possess an intrinsic biological activity being used in some cases as anticancer agents [21].

Intriguing results were observed with the platinum complexes. Indeed, complexes **3b** and **3c** were inactive on all cells tested showing an IC_{50} > 25 μM in each case. These results can be explained by two main factors; the low solubility of the complexes and the unsuitable complexation pattern obtained with a tridentate ligand. On the other hand, the classic platinum(II) complex **3a** displays very interesting antiproliferative activities. Indeed, it possesses an IC_{50} of 2.2 μM which is essentially equal to that of CDDP displaying an IC_{50} of 2.1 μM on AR+ PCa cell LNCaP. Even more interesting, complex **3a** shows selectivity towards the AR+ PCa cell line. In fact, it is about 4 to 6 times more potent on LNCaP cell in comparison to the AR- PCa cells: DU145 (IC_{50} of 8.8 μM) and PC3 (13.3 μM). On the latter two types of cells, CDDP showed an IC_{50} of 1.0 μM and 0.5 μM, respectively. Hence, no selectivity was observed with CDDP on any type of cells, its inhibitory concentration varying from 0.5 to 5.1 μM on the cell tested. It is worth noting that, not only the testosterone-platinum(II) complex **3a** is selective on hormone-dependent PCa cells but, it also displays low activity on primary human immortalized keratinocyte cells (HaCat) as well as on the colon carcinoma (HT-29) with IC_{50} > 25 μM in both cases.

3. Conclusion

In this study, we prepared and characterized several testosterone-platinum(II) hybrid molecules designed for the treatment of PCa. Two types of hybrids were made: hybrid **3a** constructed with a bidentate ligand and two hybrids **3b** and **3c** synthesized with tridentate ligands. They were prepared by efficient transformation of testosterone into an azide derivative **5** (37% overall) which was reacted with pyridine-alkyne derivatives via a cycloaddition reaction to give the precursor 1,2,3-triazole ligands **6a-c** with 48% average yield. Complexation occurred upon treatment with potassium tetrachloroplatinate to give the final hybrids **3a-c** with 57% average yield. Antiproliferative assays showed that the ligands possess intrinsic biological activities on the cells tested exhibiting IC_{50} ranging from 6.3 to 20 μM at the exception of ligand **6c** with IC_{50} > 25 μM on LNCaP cells. Unfortunately, the platinum(II) complexes **3b** and **3c** had low solubility and were found to be completely inactive at the maximum dose tested. However, the platinum(II) complex **3a** exhibited promising *in vitro* selectivity on AR+ PCa cells LNCaP, being from 4 and 6 times more active than on the AR- DU145 and PC3 cancer cells. Furthermore, this hybrid was inactive on primary human immortalized keratinocyte cells (HaCaT) as well as on colon carcinoma (HT-29). Despite the fact that the hybrids **3b** and **3c** were inactive, the precursor ligands give surprising results and could potentially be used as antiandrogens. Further study are needed to confirm this hypothesis. In conclusion, it is recommended to design classic platinum(II) hybrid molecules such as **3a** in order to obtain products with significant antiproliferative activity and selectivity on AR+ PCa.

4. Experimental protocols

4.1 Biological Methods

The antiproliferative activity assay of all compounds was assessed using the procedure recommended by the National Cancer Institute for its drug-screening program with minor modifications [22]. LNCaP androgen-sensitive human prostate adenocarcinoma, PC3 androgen-insensitive human prostate adenocarcinoma, DU145 androgen-insensitive human prostate adenocarcinoma, HT-29 human colon carcinoma and HaCaT primary human immortalized keratinocyte cells were purchased from the American Type Culture Collection (Manassas, VA). LNCap, PC3, DU145 and HT29 were cultured in RPMI medium (Hyclone, Logan, UT) supplemented with 10% of calf serum and Penicillin-Streptomycin-Glutamine. HaCat were cultured in DMEM medium supplemented with 10% FBS and Penicillin-Steptomycin-Glutamine. The cells were maintained at 37 °C in a moisture saturated atmosphere containing 5% CO_2.

4.1.1 Antiproliferative activity assay

The antiproliferative activity of compounds **3a-c**, **6a-c** and CDDP was assessed using the SRB assay [18,19]. Briefly, the 96-well microtiter plates were seeded with 75 µL of cells suspended (LNCaP, 12 x 10^3; PC3, 6 x 10^3; DU 145, 6 x 10^3; HT-29, 3 x 10^3; HaCat, 5 x 10^3) in medium. Plates were incubated at 37 °C, 5% CO_2 for 24 h. Freshly solubilized drugs in DMF were diluted in fresh medium, and 75 µL aliquots containing serially diluted concentrations of the drug were added and the plates were incubated for 72 h. Final drug concentrations ranged from 25 µM to 0.780 µM. The final concentration of DMF in the culture media was 0.1% and was kept constant in all experiments. Plates containing attached cells were then stained with sulforhodamine B to determine the number of viable cells. Briefly, cells were fixed by addition of cold trichloroacetic acid to the wells (10% (w/v) final concentration), for 30 minutes at 4 °C. Plates were washed five

times with tap water and dried. Sulforhodamine B solution (75 μL) at 0.1% (w/v) in 1% acetic acid was added to each well, and the plates were incubated for 15 minutes at room temperature. Unbound dye was removed by washing five times with 1% acetic acid. Bonded dye was solubilized in 10 mM Tris base, and the absorbance was read using a μQuant Universal microplate spectrophotometer (Biotek, Winooski, VT) at a wavelength between 530 and 565 nm according to color intensity. Readings obtained from treated cells were compared with measurements from control cells plates fixed on the treatment day, and the percentage of cell growth inhibition was then calculated for each drug. The experiments were performed three times in triplicate at the exception of the LNCaP cells which was performed once in triplicate. The assays were considered valid when the coefficient of variation for a given set of conditions and within the same experiment was < 10%.

4.2 Chemistry

Anhydrous reactions were performed under an inert atmosphere of nitrogen. The starting material, reactant and solvents were obtained commercially and were used as such or purified and dried by standard means [23]. Organic solutions were dried over magnesium sulfate ($MgSO_4$), filtered and evaporated on a rotary evaporator under reduced pressure. All reactions were monitored by UV fluorescence. Commercial TLC plates were Sigma T 6145 (polyester silica gel 60Å, 0.25mm). Flash column chromatography was performed according to the method of Still et al. on Merck grade 60 silica gel, 230-400 mesh [24]. All solvents used in chromatography were distilled prior to use.

The infrared spectra were taken on a Nicolet Impact 420 FT-IR spectrophotometer. Mass spectral assays were obtained using a MS model 6210, Agilent technology instrument. The high resolution mass spectra (HRMS) were obtained by TOF (time of flight) using ESI (electrospray ionization) using the positive mode (ESI+) (Université du Québec à Montréal and at Université Laval). Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 200 MHz NMR apparatus. Samples were dissolved in deuterated chloroform ($CDCl_3$) or in the cases of the platinum complexes in a mixture of deuterated chloroform and methanol ($CDCl_3$ + CD_3OD ≈ 2 drops) for data acquisition using the residual solvent signal as internal standard (chloroform, δ 7.26 ppm for ^1H NMR and δ 77.23 ppm for ^{13}C NMR). Chemical shifts (δ) are expressed in parts per million (ppm), the coupling constants (J) are expressed in hertz (Hz). Multiplicities are described by the following abbreviations: s for singlet, d for doublet, t for triplet and m for multiplet and, bs for broad singlet.

Note: The nomenclature of the testosterone derivatives reported herein was based on the androgen skeleton (4-androsten-17β-ol-3-one) for clarity to the readers. This should facilitate the visualization of the molecular structures, particularly for researchers working with steroids.

4.2.1 Synthesis of testosterone-platinum(II) hybrids

4.2.1.1 Synthesis of 7α-(4-chloro-but-2-enyl)-4-androsten-17β-ol-3-one (4)

The 7α-allyl-4-androstene-17β-ol-3-one acetate (842 mg, 2.01 mmol, 1 eq.) is dissolved in methanol (16 mL) and the solution is stirred. 5 N HCl solution (5 mL) is added to the mixture and stirred for 48 hours at room temperature. The solution is then diluted with ether (50 mL) and the

organic layer is then washed with saturated $NaHCO_3$ solution (50 mL). The organic layer is then washed 3 times with distilled water (25 mL), dried over $MgSO_4$, filtered and evaporated. The product is purified by flash chromatography (hexane/acetone, 8:2) to give 640 mg of **4**. Yield, 95 %; white solid; mp: 142 – 145 °C. IR ν (cm^{-1}): 3423 (O-H), 1669 (C=O, enone), 1614 (C=C), 1250 (C-O). ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.67 (1H, s, 4-CH), 5.56 (2H, m, 21-CH and 22-CH), 3.98 (2H, m, 23-CH_2), 3.62 (1H, t, J=8.2 Hz, 17-CH), 1.17 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3).

4.2.1.2 Synthesis of 7α-(4-azido-but-2-enyl)-4-androsten-17β-ol-3-one (5)

Steroid **4** (218 mg, 0.58 mmol, 1 eq.) is dissolved in methanol (10 mL). Sodium azide (226 mg, 3.47 mmol, 6 eq.) is added to the solution, stirred and heated (reflux) for 2 hours. Let the mixture cool down to room temperature. A liquid-liquid extraction is performed with a mix of ether and ether (8:2). The organic layer is extracted 3 portions of water (25 mL) and one portion of saturated NaCl solution (25 mL). The organic phase is then dried over $MgSO_4$, filtered and evaporated under pressure to give 204 mg of the desired material. No purification needed for this compound. Yield, 92 %; beige solid; mp: 139 – 143 °C. IR ν (cm^{-1}): 3432 (O-H), 2945, 2915 and 2873 (C-H), 2094 and 2073 (N=N=N), 1653 (C=O), 1617 (C=C), 1239 (C-O), 875 (C-Cl). ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 5.72 (1H, s, 4-CH), 5.56 (2H, m, 21-CH and 22-CH), 3.67 (3H, m, 23-CH_2 and 17-CH), 1.21 (3H, s, 19-CH_3), 0.80 (3H, s, 18-CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ ppm): 199.1 (C-3), 169.1 (C-5), 134.9 (C-21), 126.2 (C-22), 125.1 (C-4), 81.6 (C-17), 52.6, 47.2, 46.2, 42.9 (C-23), 38.9, 38.8, 36.9, 36.5, 36.4, 36.2, 34.2, 30.5, 28.8, 23.0, 21.1, 18.2, 11.2. HRMS (ESI+): $(M+H)^+$ calculated for $C_{23}H_{34}N_3O_2$ = 384.2646; found : 384.2662.

4.2.1.3 Synthesis of prop-2-ynyl-pyridin-2-ylmethylamine (7b)

2-picolylamine (1.0 mL, 9.70 mmol, 2 eq.) and K_2CO_3 (1.34 g, 9.70 mmol, 2 eq.) are dissolved in CH_3CN (5 mL) and stirred for 10 minutes. Propargyl bromide (430 µL, 4.85 mmol, 1 eq.), diluted in CH_3CN (25 mL), is added dropwise to the mixture over 2 hours and then stirred for 16 hours at room temperature. The reaction is then filtered to eliminate K_2CO_3 and the solvent is evaporated. The product is purified by flash chromatography (DCM/methanol/TEA, 100:5:1) to give **7b** (355 mg). Yield, 50 %; orange oil. IR ν (cm^{-1}): 3293 (NH secondary), 1674, 1590, 1569, 1474, 1433, 1357, 1148, 1121. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 8.55 (1H, d, J=4.7 Hz, a-CH), 7.62 (1H, td, J=7.8 Hz 1.8 Hz, c-CH), 7.30 (1H, d, J=7.8 Hz, d-CH), 7.14 (1H, m, b-CH), 3.98 (2H, s, pyr-$\underline{CH_2}$-NH-CH_2), 3.48 (2H, d, J= 2.3 Hz, pyr-CH_2-NH-$\underline{CH_2}$), 2.23 (1H, t, J=2.5 Hz, CH alkyne), 2.12 (1H, bs, NH). ^{13}C NMR (200 MHz, $CDCl_3$, δ ppm): 159.0, 149.3, 136.4, 122.4, 122.0, 81.8, 71.7, 53.6, 37.7. HRMS (ESI+): $(M+H)^+$ calculated for $C_9H_{11}N_2$ = 147.0917; found : 147.0920.

4.2.1.4 Synthesis of prop-2-ynyl-pyridin-2-ylethylamine (7c)

2-(2-aminoethyl)pyridine (1.0 mL, 8.40 mmol, 2 eq.) and K_2CO_3 (1.16 g, 8.41 mmol, 2 eq.) are dissolved in CH_3CN (5 mL) and stirred for 10 minutes. Propargyl bromide (370 µL, 4.2 mmol, 1 eq.), diluted in CH_3CN (25 mL), is added dropwise to the mixture over 2 hours and then stirred for 16 hours at room temperature. The reaction is then filtered to eliminate K_2CO_3 and the solvent is evaporated. The product is purified by flash chromatography (DCM/methanol/ Et_3N, 100:5:1) to give **7c** (309 mg). Yield, 40 %; orange oil. IR ν (cm^{-1}): 3294 (NH), 1674, 1591, 1568, 1474, 1433, 1116, 749, 629. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 8.51 (1H, d, J=4.7 Hz, a-CH), 7.59 (1H, td, J=7.8 Hz and 2.0 Hz, c-CH), 7.17 (1H, d, J=7.8 Hz, d-CH), 7.10 (1H, m, b-CH), 3.44 (2H, d, J=2.7 Hz, CH_2CH_2-NH-$\underline{CH_2}$), 3.10 (2H, m, $\underline{CH_2CH_2}$-NH-CH_2), 2.96 (2H, m, $\underline{CH_2}CH_2$-

NH-CH_2), 2.18 (1H, t, J=2.5 Hz, CH alkyne), 1.81 (1H, bs, NH). ^{13}C NMR (200 MHz, $CDCl_3$, δ ppm): 159.9, 149.2, 136.3, 123.2, 121.3, 82.0, 71.4, 48.0, 38.0 (2 CH_2). HRMS (ESI+): $(M+H)^+$ calculated for $C_{10}H_{13}N_2$ = 161.1073; found : 161.1077.

4.2.1.5 Synthesis of 7α-[4-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-but-2-enyl]-4-androsten-17β-ol-3-one (6a)

Azide **5** (84 mg, 0.22 mmol, 1 eq.) and 2-ethynylpyridine (**9a**) (37 µL, 0.37 mmol, 1.7 eq.) are added to a mixture of THF/water (3:1, 6 mL) and stirred. Then, sodium ascorbate (0.55 mmol, 2.5 eq.) and $CuSO_4 \cdot 5\ H_2O$ (16.4 mg, 0.07 mmol, 0.3 eq.) are added and the mixture is stirred at room temperature for 16 hours. The product is extracted with EtOAc (50 mL) and washed thrice with water (3 x 30 mL). The organic phase is dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product is purified by flash chromatography (hexanes/acetone, 7:3) to give **6a** (57 mg). Yield, 54 %; brownish oil. IR ν (cm^{-1}): 3391 (O-H), 3150 (C-H triazole), 2944 (C-H), 1610 (C=O), 1570 (C=N), 1436, 1355, 1225, 950, 785. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 8.56 (1H, d, J=5.2 Hz, a-CH), 8.17 (1H, d, J=7.8 Hz, d-CH), 8.13 (1H, s, 24-CH-triazole), 7.77 (1H, td, J=7.8 Hz and 1.6 Hz, c-CH), 7.21 (1H, m, b-CH), 5.68 (3H, bs, 4-CH, 21-CH and 22-CH), 4.98 (2H, m, 23-CH_2), 3.65 (1H, t, J=8.0 Hz, 17-CH), 1.20 (3H, s, 19-CH_3), 0.79 (3H, s, 18-CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ ppm): 198.9, 168.9, 150.2, 149.3, 148.5, 136.9, 135.5, 126.1, 124.9, 122.9, 121.7, 120.3, 81.5, 52.1, 47.1, 46.2, 42.9, 38.7, 38.5, 36.3, 36.2, 36.1, 35.9, 33.9, 30.2, 28.6, 22.8, 20.8, 17.9, 10.9. HRMS (ESI+): $(M+H)^+$ calculated for $C_{30}H_{39}N_4O_2$ = 487.3068; found : 487.3080.

4.2.1.6 Synthesis of 7α-[4-(4-(((pyridin-2-ylmethyl)-amino)-methyl)-[1,2,3]-triazol-1-yl)-but-2-enyl]-4-androsten-17β-ol-3-one (6b)

Azide **5** (55 mg, 0.14 mmol, 1 eq.) and alkyne **7b** (42 mg, 0.29 mmol, 2 eq.) are added to a mixture of THF/water (1:1, 1.3 mL) and stirred. Then, sodium ascorbate (5.7 mg, 0.029 mmol, 0.2 eq.) and $CuSO_4 \cdot 5\ H_2O$ (3.6 mg, 0.015 mmol, 0.1 eq.) are added and the mixture is stirred at room temperature for 16 hours. The product is extracted with EtOAc (50 mL) and washed twice with saturated NaCl solution (2 x 30 mL). Aqueous phases are combined and reextracted with EtOAc. Organic phases are combined and dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product is purified by flash chromatography (DCM/methanol/Et_3N, 100:5:1) to give **6b** (22 mg). Yield, 29 %; orange oil. IR ν (cm^{-1}): 3304 (O-H), 2939 (C-H), 2094, 2073, 1654 (C=O), 1611 (C=C). ^1H NMR (200 MHz, $CDCl_3$, δ ppm): 8.54 (1H, m, a-CH), 7.65 (1H, m, c-CH), 7.56 (1H, s, 24-CH), 7.35 (1H, m, d-CH), 7.18 (1H, m, b-CH), 5.60 (3H, bs, 4-CH, 21-CH and 22-CH), 4.89 (2H, m, 23-CH_2), 4.03 (4H, m, 26-CH_2 and 27-CH_2), 3.64 (1H, t, J=8.2 Hz, 17-CH), 1.19 (3H, s, 19-CH_3), 0.79 (3H, s, 18-CH_3).^{13}C NMR (200 MHz, $CDCl_3$, δ ppm): 199.0, 169.0, 158.7, 149.3, 146.4, 136.6, 135.2, 126.1, 125.1, 122.5, 122.2, 121.7, 81.5, 51.9, 47.1, 46.1, 44.1, 42.9, 38.7, 38.5, 36.4, 36.2, 36.0, 35.9, 33.9, 30.2, 29.7, 28.8, 22.8, 20.8, 17.9, 10.9. HRMS (ESI+): $(M+H)^+$ calculated for $C_{32}H_{44}N_5O_2$ = 530.3490; found : 530.3505.

4.2.1.7 Synthesis of 7α-[4-(4-((2-pyridin-2-yl-ethylamino)-methyl)-[1,2,3]-triazol-1-yl)-but-2-enyl]-4-androsten-17β-ol-3-one (6c)

Azide **5** (56 mg, 0.15 mmol, 1 eq.) and alkyne **7c** (25 mg, 0.16 mmol, 1.1 eq.) are added to a mixture of *t*-BuOH/water (1:1, 2 mL) and stirred. Then, sodium ascorbate (5.8 mg, 0.029 mmol, 0.2 eq.) and $CuSO_4 \cdot 5\ H_2O$ (3.6 mg, 0.015 mmol, 0.1 eq.) are added and the mixture is stirred at room temperature for 16 hours. The product is extracted with EtOAc (50 mL) and washed twice with saturated NaCl solution (2 x 30 mL). Aqueous phases are combined and reextracted with

EtOAc. Organic phases are combined and dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product is purified by flash chromatography (DCM/methanol/Et_3N, 100:5:1) to give 48 mg of **6c**. Yield, 60 %; light brown oil. IR ν (cm^{-1}): 3306 (O-H and N-H), 2936 (C-H), 1659 (C=O), 1613 (C=C), 1592, 1374, 1222, 1138, 1051, 750. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 8.47 (1H, d, J=4.7 Hz, a-CH), 7.56 (1H, td, J=7.8 Hz and 1.8 Hz, c-CH), 7.48 (1H, s, 24-CH-triazole), 7.10 (2H, m, b-CH and d-CH), 5.58 (3H, bs, 4-CH, 21-CH and 22-CH), 4.85 (2H, m, 23-CH_2), 3.93 (2H, s, 26-CH_2), 3.61 (1H, t, J=8.2 Hz, 17-CH), 3.01 (4H, m, 27-CH_2 and 28-CH_2), 1.22 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ ppm): 198.9, 168.9, 159.9, 149.2, 146.5, 136.5, 135.2, 126.1, 125.1, 123.3, 121.5, 121.4, 81.4, 51.9, 48.7, 47.1, 46.1, 44.5, 42.9, 38.7, 38.5, 36.4, 36.2, 36.0, 35.9, 33.9, 30.2, 29.7, 28.7, 22.8, 20.8, 17.9, 10.9. HRMS (ESI+): $(M+H)^+$ calculated for $C_{33}H_{46}N_5O_2$ = 544.3646; found : 544.3662.

4.2.1.8 Synthesis of 7α-[4-(4-pyridin-2-yl-[1,2,3]triazol-1-yl)-but-2-enyl]-4-androsten-17β-ol-3-one dichloroplatinum(II) (3a)

Triazole compound **6a** (57 mg, 0.117 mmol, 1 eq.) is added to 1.0 mL of DMF and stirred for several minutes. Potassium tetrachloroplatinate(II) (K_2PtCl_4) (51 mg, 0.12 mmol, 1.1 eq.) is diluted in 0.5 mL of distilled water and added to the solution of compound **6a**. The mixture is stirred for 5 days in the dark. After 5 days, 2 mL of a saturated KCl solution and 0.2 g of solid KCl is added to the mixture and stirred vigorously overnight (16 hours). The product is filtered and washed with distilled water. The product is purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give **3a** (31 mg). Yield, 34 %; yellow solid, mp: 255 – 257 °C. IR ν (cm^{-1}): 3403 (N-H and OH), 3103, 2931 (C-H), 1653 (C=O), 1623 (C=C), 1463, 1356, 1274, 1052, 985, 775. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 9.08 (1H, m, a-CH), 8.55 (1H, s, 24-CH),

8.05 (2H, m, c-CH and d-CH), 7.26 (1H, m, b-CH), 5.92 (1H, m, 4-CH), 5.65 (2H, m, 21-CH and 22-CH), 5.12 (2H, m, 23-CH_2), 3.62 (1H, t, J=9.0 Hz, 17-CH), 1.19 (3H, s, 19-CH_3), 0.78 (3H, s, 18-CH_3). ^{13}C NMR (200 MHz, $CDCl_3$ + CD_3OD (9 :1), δ ppm): 199.9, 170.4, 149.4, 148.4, 139.7, 139.1, 137.0, 125.8, 124.9, 122.8, 122.4, 81.1, 54.8, 50.6, 50.1, 47.1, 46.1, 42.8, 38.7, 38.5, 36.5, 36.1, 35.8, 33.9, 29.8, 28.9, 22.7, 20.8, 17.9, 10.9. HRMS (ESI+): $(M+H)^+$ calculated for $C_{30}H_{42}Cl_2N_5O_2Pt$ = 770.2348; found : 770.2376.

4.2.1.9 Synthesis of 7α-[4-(4-(((pyridin-2-ylmethyl)-amino)-methyl)-[1,2,3]-triazol-1-yl)-but-2-enyl]-4-androsten-17β-ol-3-one chloroplatinum(II) chloride (3b)

Triazole compound **6b** (34.2 mg, 0.06 mmol, 1 eq.) is added to 1.0 mL of DMF and stirred for several minutes. Potassium tetrachloroplatinate(II) (K_2PtCl_4) (28 mg, 0.07 mmol, 1.1 eq.) is diluted in 0.5 mL of distilled water and added to the solution of compound **6b**. The mixture is stirred for 5 days in the dark. After 5 days, 2 mL of a saturated KCl solution and 0.2 g of solid KCl is added to the mixture and stirred vigorously overnight (16 hours). The product is filtered and washed with distilled water to give **3b** (37.4 mg). Yield, 77 %; beige solid, mp: degrades at 256 °C. IR ν (cm^{-1}): 3438 (O-H and N-H), 3080, 2931 (C-H), 2360 – 2154 (N-N=N), 1653 (C=O), 1614 (C=C), 1442, 1348, 1240, 1056, 950, 771. ^{1}H NMR (200 MHz, $CDCl_3$ + CD_3OD (9 :1), δ ppm): 8.82 (1H, m, a-CH), 7.96 (2H, m, 24-CH and c-CH), 7.51 (1H, m, d-CH), 7.35 (1H, m, b-CH), 5.82 – 5.45 (3 H, m, 4-CH, 21-CH and 22-CH), 4.91 (2H, m, 24-CH_2), 4.59 (2H, m, CH_2), 4.22 – 4.00 (2H, m, CH_2), 3.49 (1H, t, 17-CH), 1.12 (3H, s, 19-CH_3), 0.69 (3H, s, 18-CH_3). HRMS (ESI+): $(M+H)^+$ calculated for $C_{32}H_{43}ClN_5O_2Pt$ = 760.2747; found : 760.2771.

4.2.1.10 Synthesis of 7α-[4-(4-((2-(pyridin-2-yl-ethylamino)-methyl)-[1,2,3]-triazol-1-yl)-but-2-enyl]-4-androstene-17β-ol-3-one chloroplatinum(II) chloride (3c)

Triazole derivative **6c** (53 mg, 0.1 mmol, 1 eq.) is added to 1.5 mL of DMF and stirred for several minutes. Potassium tetrachloroplatinate(II) (K_2PtCl_4) (42.5 mg, 0.1 mmol, 1 eq.) is diluted in 0.75 mL of distilled water and added to the solution of compound **6c**. The mixture is stirred for 5 days in the dark. After 5 days, 2 mL of a saturated KCl solution and 0.2 g of solid KCl is added to the mixture and stirred vigorously overnight (16 hours). The product is filtered and washed with distilled water to give **3c** (45 mg). Yield, 60 %; beige solid, mp: degrades at 253 °C. IR ν (cm^{-1}): 3413 (N-H and O-H), 3120, 2925 and 2854 (C-H), 2361 – 2160, 1653 (C=O), 1612 (C=C), 1446, 1352, 1272, 1020, 960, 747. ^{1}H NMR (200 MHz, $CDCl_3$ + CD_3OD (9 :1), δ ppm): 9.14 (1H, d, J=6.3 Hz, a-CH), 8.09 (1H, s, 24-CH), 7.87 (1H, t, J=7.2 Hz, c-CH), 7.38 (2H, m, b-CH and d-CH), 5.75 – 5.51 (3H, m, 4-CH, 21-CH and 22-CH), 5.01 (2H, m, 23-CH_2), 4.10 – 3.83 (2H, m, 26-CH_2), 3.59 (1H, t, J=8.2 Hz, 17-CH), 3.38 – 2.84 (4H, m, 27-CH_2 and 28-CH_2), 1.17 (3H, s, 19-CH_3), 0.76 (3H, s, 18-CH_3). HRMS (ESI+): $(M+H)^+$ calculated for $C_{33}H_{45}ClN_5O_2Pt$ = 774.2904; found : 774.2928.

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Legend of Figure and Scheme

Fig. 1. Structure of testosterone-anticancer hybrids **1**, **2** and **3** designed in our laboratory.

Scheme 1

Reagents and conditions: (i) NaN_3, CH_3OH, reflux, 2 h (92%), (ii) 1. Relevant pyridine-acetylene, sodium ascorbate, copper sulfate, THF:H_2O (3:1), 22 °C, 16 h (48% average yield). (iii) K_2PtCl_4, DMF:H_2O (2:1), 22 °C, 5 days (57% average yield).

Scheme 2

Reagents and conditions: i) 1. 2-Picolylamine or 2-(2-aminoethyl)pyridine, K_2CO_3, CH_3CN, addition of propargyl bromide over 2 h at 22 °C; 2. Stirring at 22°C for 16 h (48% average yield).

Table 1

Antiproliferative activity of novel testosterone-1,2,3-triazole-pyridine ligands (compounds **6a-c**) and testosterone-platinum(II) hybrids (compounds **3a-c**) and CDDP on androgen-sensitive (LNCaP) and androgen-insensitive (PC3 and DU145) human prostate adenocarcinoma cell lines and on colon carcinoma (HT-29) and normal human adult keratinocyte cells (HaCat).

Compound	**IC_{50} (μM)[a]**				
	LNCaP (AR^+)	PC3 (AR^-)	DU145 (AR^-)	HT-29	HaCat
6a	6.3	11.0	12.3	19.9	18.0
6b	20.0	13.3	13.8	14.7	18.3
6c	>25	10.5	14.4	8.5	16.4
3a	2.2	13.3	8.8	>25	>25
3b	>25	>25	>25	>25	>25
3c	>25	>25	>25	>25	>25
CDDP	2.1	0.5	1.0	1.3	5.1

[a] Inhibitory concentration (IC_{50}) is concentration of drug inhibiting cell growth by 50%. Cisplatin (CDDP)

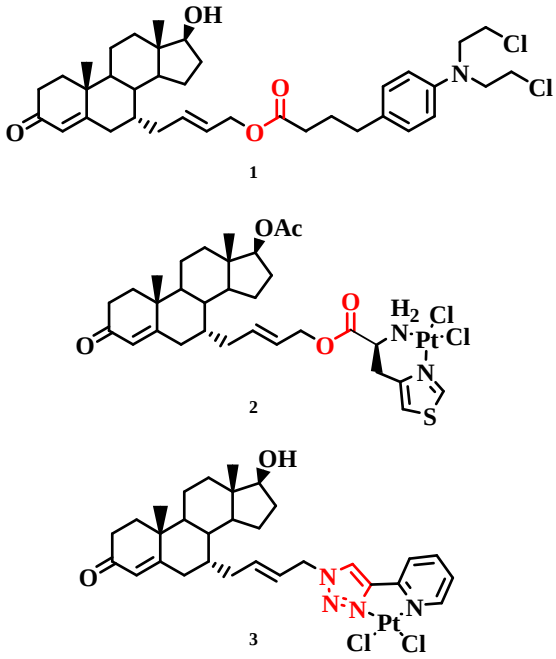
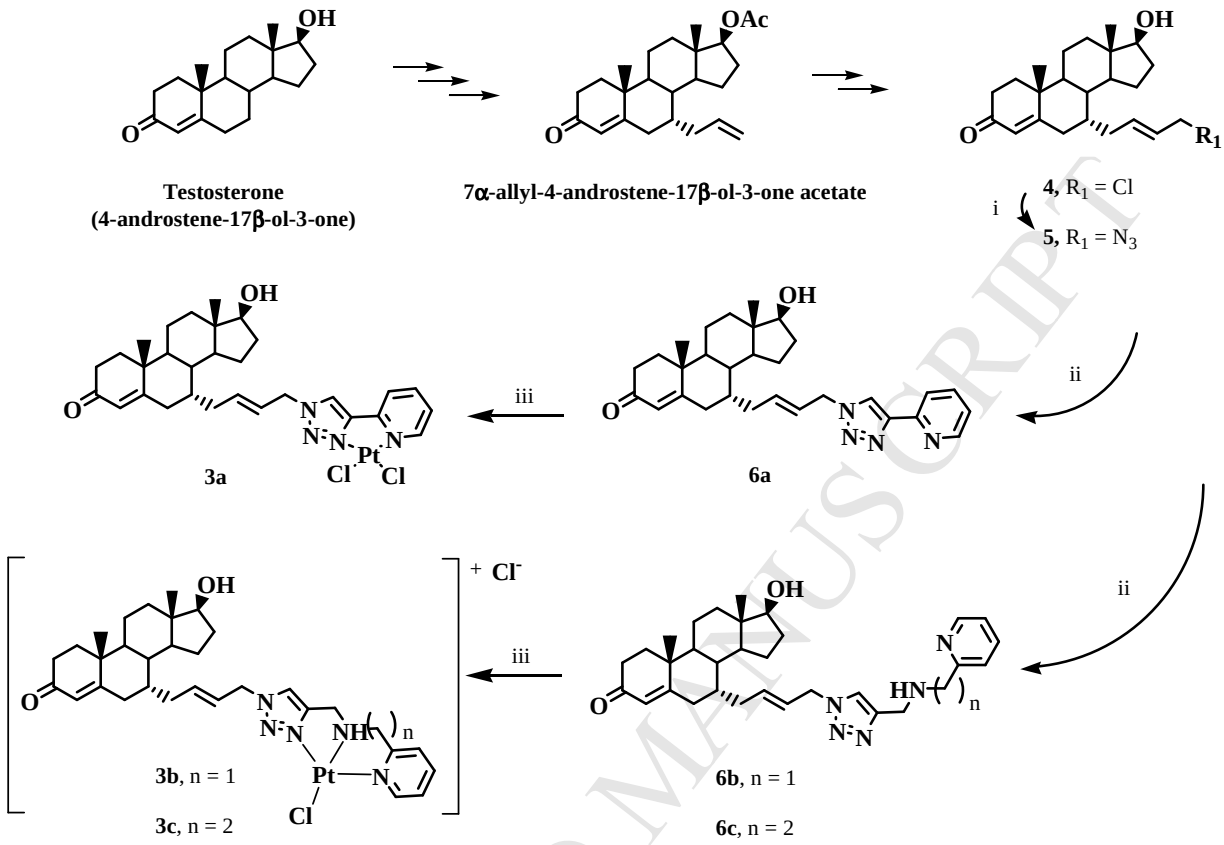
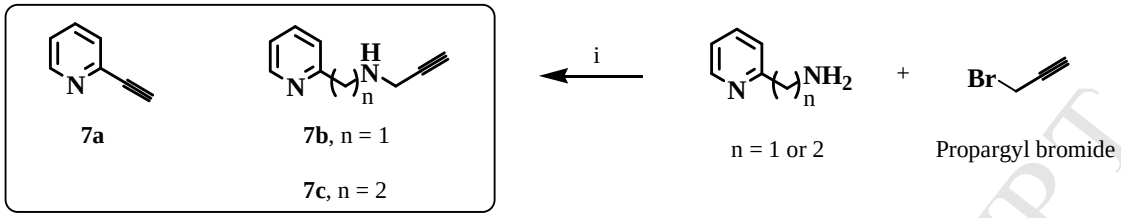


Fig. 1.



Scheme 1



Scheme 2

Highlights

- Novel testosterone-platinum(II) hybrids are reported.
- The best hybrid exhibits IC_{50} in of 2.2 μM on AR+ prostate cancer cells (LNCaP).
- The best hybrid was selective on AR+ LNCaP cells and was as active as cisplatin.
- The best hybrid was inactive on normal human adult keratinocyte cells (HaCaT).
- The precursor ligands showed significant antiproliferative activity.