**Short Note**

**Dichloro[N-(n^6-phenyl)methyl]-4-(1-(3,5,5,8,8-pentamethyl- 5,6,7,8tetrahydronaphthalen-2-yl)vinyl]benzamide] (1,3,5-triaza-7-phosphatricyclo[3.3.1.1^{3,7}]decane-κP^{7}) ruthenium**

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**Abstract:** Bexarotene-tethered Ru(II)(arene) compounds with 1,3,5-triaza-7-phosphatricyclo[3.3.1.1^{3,7}] decane (PTA) were prepared as an analog of RAPTA antitumor complexes in order to evaluate their in vitro antiproliferative activity against human cancer cell lines.

**Keywords:** ruthenium compounds; bexarotene; PTA ligand; antiproliferative activity

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1. **Introduction**

Ruthenium-based antitumor drugs are the most widely studied after platinum-based complexes [1–3] and are first-in-class approved by FDA as an orphan drug against gastric cancer BOLD 100 (Figure 1) [4]. OrganorutheniumII(arene) compounds with PTA co-ligands, termed RAPTA (Figure 1), have previously shown excellent tumor-inhibiting properties [5].

![Figure 1. The structure of BOLD-100, RAPTA and Bexarotene.](image_url)

Tethering the known metal-based drugs with the biologically active molecule is a promising approach in medicinal chemistry to improve cytotoxicity and selectivity [6–8]. Recently we have utilized the antitumor drug bexarotene, a selective agonist of retinoid X receptors (RXRs) and an FDA-approved drug, to treat cutaneous T cell lymphoma, improving the antitumor activity of Ru(II), Ru(III), and Pt(IV) complexes [9–11]. In this paper, we have followed the same approach and prepared RAPTA analogs with bexarotene moiety attached to the arene part of the complex. The synthesized compound was fully characterized using NMR spectroscopy and mass-spectrometry with electrospray ionization (ESI-MS). The antiproliferative activity was investigated against human cancer cell lines.

2. **Results**

The RAPTA analog with the bexarotene moiety 2 (Figure 2) was prepared by the reaction of the PTA ligand with Ru-dimer 1 in CH2Cl2 at room temperature (Scheme 1).
A pure compound was isolated by precipitation out of the reduced-in-volume reaction mixture by the petroleum ether. The compound was characterized by $^1$H, $^{13}$C($^1$H), and $^{31}$P($^1$H) NMR spectroscopy, ESI-MS, and elemental analysis (full spectra available in Supplementary Materials). $^{31}$P($^1$H) NMR spectroscopy was used to monitor the formation of the complex. These spectra included a shift from ca. −100 to −30 ppm for the PTA ligand upon coordination. The $^1$H NMR spectrum had no significant shift in the proton signals for both the arenne fragment and the CH$_2$ groups of the PTA ligand compared to the initial ruthenium dimer. ESI-mass spectra were recorded without the addition of any acid or base in both the positive and negative modes, hence, the ions corresponding to complex 2 appeared mainly due to the loss or gain of the chloride ([M − Cl]$^+$ and [M + Cl]$^-$ ions).

Figure 2. The structure of the target compound.

Scheme 1. Synthesis of RAPTA analog with bexarotene moiety.

For the obtained complex 2, the antiproliferative activity was studied by the MTT-assays on a series of human cancer cell lines (lung carcinoma A549, colon carcinoma HCT116, breast adenocarcinoma MCF7, colon adenocarcinoma SW480) and is presented in Table 1. The complex exhibits activity in the range of low micromolar concentrations, exceeding the activity of the parent drug bexarotene Ru-dimer 1 and greatly exceeding the activity of RAPTA-type complexes.

Table 1. The 50% inhibitory concentrations of complex 2, bexarotene and cisplatin. Values are means ± SDs obtained by the MTT-assay (exposure time: 72 h).

| Compounds    | A549   | HCT116 | MCF7   | SW480  |
|--------------|--------|--------|--------|--------|
| 2            | 31 ± 4 | 32 ± 7 | 27 ± 2 | 23.4 ± 0.6 |
| cisplatin    | 8.8 ± 0.9 | 12 ± 2 | 13 ± 1 | 6.2 ± 0.6 |
| Bexarotene   | 50 ± 2 | 51 ± 2 | 75 ± 1 | 69 ± 1 |
3. Materials and Methods

3.1. General

All commercial reagents were used without further purification. All solvents were purified and degassed before use. $^1$H NMR, $^{13}$C NMR, and $^{31}$P spectroscopy were performed at 298 K on Bruker Avance 600. $^1$H and $^{13}$C NMR spectra were calibrated against the residual solvent: CDCl$_3$. $^{1}$H and $^{13}$C NMR spectra were calibrated against the residual solvent: CDCl$_3$. $^{31}$P spectra were calibrated by external reference (85% H$_3$PO$_4$ in H$_2$O, $\delta$ = 0 ppm). The splitting of the proton resonances in the reported $^1$H spectra is defined as s = singlet, d = doublet, t = triplet, and m = multiplet. Mass-spectra were recorded using TSQ Endura (Thermo Scientific, Waltham, MA, USA) mass-spectrometer with an electrospray ionization source (ESI). The methanol solution of each compound was introduced through a syringe pump directly into the ion source at 5–10 $\mu$L/min. Massspectra were acquired in both positive and negative modes. The system was controlled by the Xcalibur software, which was also used for data collection and data processing. The ion transfer tube temperature was set to 275 $^\circ$C, and the vaporizer temperature to 40 $^\circ$C. The Sheath and Aux gases were 6 and 5 units, while the spray voltage was 3.4 and 2.5 kV for both positive and negative modes correspondingly. The spectra were recorded during 30 s in the $m/z$ range 150–1400.

Dichloro[N-[(η$^6$-phenyl)methyl]-4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)vinyl]benzamide][1,3,5-triaza-7-phosphatricyclo[3.3.1.1$^3$]decane-$\kappa$P$_7$]ruthenium.

A solution of 1,3,5-triaza-7-phosphaadamantane (26 mg, 0.16 mmol) in CH$_2$Cl$_2$ (1 mL) was added to a solution of ruthenium dimer 1 (100 mg, 0.08 mmol) in CH$_2$Cl$_2$ (14 mL). The reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated under the vacuum to a minimum volume, the product was precipitated with petroleum ether, separated by centrifugation, and the resulting dark orange powder was dried in a vacuum.

Yield 107 mg (85%, $T_{dec}$ = 230-235 $^\circ$C)

$^1$H NMR (600.13 MHz, CDCl$_3$): $\delta$(ppm) 8.08 (t, 1H, $J$ = 5.4 Hz, NH), 7.88 (d, 2H, $J$ = 8.0 Hz, H16; H18), 7.37 (d, 2H, $J$ = 8.0 Hz, H15; H19), 7.12 (s, 1H, H6), 7.07 (s, 1H, H3), 5.80 (s, 1H, H13), 5.70–5.64 (m, 4H, H27; H28; H30; H31), 5.30 (s, 1H, H13), 5.14 (t, 1H, $J$ = 4.8 Hz, H29), 4.65 (d, 2H, $J$ = 5.7 Hz, H13), 4.32 (s, 6H, H$_1$PTA; H$_2$PTA; H$_3$PTA), 1.94 (s, 3H, H14), 1.70 (s, 4H, H8; H9), 1.29 (d, 12H, $J$ = 17.2 Hz, H23; H24; H25; H26).

$^{13}$C NMR (150.92 MHz, CDCl$_3$): $\delta$(ppm) 166.7 (C$_{O}$), 148.6 (C11), 144.2 (C12), 143.8 (C2), 141.8 (C1), 137.5 (C5), 132.2 (C4), 131.3 (C17), 127.5 (C3;C6), 127.0 (C16; C18), 126.4 (C15; C19), 116.0 (C13), 100.0 (C$_{arene}$), 86.3 (C$_{arene}$), 85.0 (C$_{arene}$), 72.8 (J = 6.0 Hz, C$_4$PTA; C$_5$PTA; C$_6$PTA), 52.5 (J = 16.6 Hz, C1PTA; C2PTA; C3PTA), 40.7 (C22), 34.7 (C8; C9), 33.5 (C7/C10), 33.4 (C7/C10), 31.5 (C23/C24/C25/C26), 31.4 (C23/C24/C25/C26), 19.5 (C14).

$^{31}$P NMR (242.94 MHz, CDCl$_3$): $\delta$(ppm) $-30.2$.

Elemental analysis calculated for C$_{37}$H$_{47}$Cl$_2$N$_4$OPRu: C 57.96, H 6.18, N 7.31, found: C 58.42, H 6.07, N 7.18.

ESI-MS: $m/z$: 731 [M − Cl]$^+$, 801 [M + Cl]$^-$.

3.2. Cells and In Vitro Antiproliferative Assays

The human HCT116 colorectal carcinoma, SW480 colon adenocarcinoma, A549 non-small cell lung carcinoma, and MCF7 breast adenocarcinoma cell lines were obtained from the European collection of authenticated cell cultures (ECACC; Salisbury, UK) All cells were grown in a DMEM medium (Gibco™, Dublin, Irland) supplemented with 10% fetal bovine serum (Gibco™, Brazil). The cells were cultured in an incubator at 37 $^\circ$C in a humidified 5% CO$_2$ atmosphere and were sub-cultured 2 times a week. The effect of the investigated compounds on cell proliferation was evaluated using a common MTT assay. The cells were seeded in 96-well tissue culture plates («TPP», Trasadingen, Switzerland) at a $1 \times 10^4$ cells/well in 100 $\mu$L of the medium. After overnight incubation at 37 $^\circ$C, the
cells were treated with the tested compounds in the concentration range of 0 to 200 µM. Cisplatin was used as a standard. After 72 h of treatment, the solution was removed, and a freshly diluted MTT solution (100 µL, 0.5 mg/mL in cell medium) was added to the wells, and the plates were further incubated for 50 min. Subsequently, the medium was removed, and the formazan product was dissolved in 100 µL of DMSO. The number of living cells in each well was evaluated by measuring the absorbance at 570 nm using the «Zenith 200 rt» microplate reader (Biochrom, Cambridge, UK).

4. Conclusions

The synthesis and characterization of bexarotene—tethered RuII (arene) compounds with 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA) as an analog of the RAPTA complex has been reported here for the first time. The antiproliferative activity of the compound 2 showed the potential applications of this compound as an antitumor agent.

Supplementary Materials: The following supporting information can be downloaded. Copies of 1H, 13C, 31P NMR and mass-spectra.

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References

1. Alessio, E. Thirty Years of the Drug Candidate NAMI-A and the Myths in the Field of Ruthenium Anticancer Compounds: A Personal Perspective. *Eur. J. Inorg. Chem.* 2017, 2017, 1549–1560. [CrossRef]

2. Nazarov, A.A.; Hartinger, C.G.; Dyson, P.J. Opening the lid on piano-stool complexes: An account of ruthenium(II)–arene complexes with medicinal applications. *J. Organomet. Chem.* 2014, 751, 251–260. [CrossRef]

3. Simpson, P.V.; Desai, N.M.; Casari, I.; Massi, M.; Falasca, M. Metal-based antitumor compounds: Beyond cisplatin. *Future Med. Chem.* 2019, 11, 119–135. [CrossRef] [PubMed]

4. FDA Grants Bold Therapeutics’ BOLD-100 an Orphan Drug Designation (ODD) in the Treatment of Gastric Cancer. Available online: https://www.prnewswire.com/news-releases/fda-grants-bold-therapeutics-bold-100-an-orphan-drug-designation-odd-in-the-treatment-of-gastric-cancer-301288416.html (accessed on 11 May 2021).

5. Murray, B.S.; Babak, M.V.; Hartinger, C.G.; Dyson, P.J. The development of RAPTA compounds for the treatment of tumors. *Coord. Chem. Rev.* 2016, 306, 86–114. [CrossRef]

6. Ang, W.H.; Khalaila, I.; Allardyce, C.S.; Juillerat-Jeanneret, L.; Dyson, P.J. Rational Design of Platinum(IV) Compounds to Overcome Glutathione-S-Transferase Mediated Drug Resistance. *J. Am. Chem. Soc.* 2005, 127, 1382–1383. [CrossRef] [PubMed]

7. Kenny, R.G.; Marmion, C.J. Toward Multi-Targeted Platinum and Ruthenium Drugs—A New Paradigm in Cancer Drug Treatment Regimens? *Chem. Rev.* 2019, 119, 1058–1137. [CrossRef] [PubMed]

8. Nazarov, A.A.; Risse, J.; Ang, W.H.; Schmitt, F.; Zava, O.; Ruggi, A.; Groessl, M.; Scopelitti, R.; Juillerat-Jeanneret, L.; Hartinger, C.G.; et al. Anthracene-Tethered Ruthenium(II) Arene Complexes as Tools To Visualize the Cellular Localization of Putative Organometallic Anticancer Compounds. *Inorg. Chem.* 2012, 51, 3633–3639. [CrossRef] [PubMed]

9. Nosova, Y.N.; Karlov, D.S.; Pisarev, S.A.; Shutkov, I.A.; Palyulin, V.A.; Baqui, M.; Milaeva, E.R.; Dyson, P.J.; Nazarov, A.A. New highly cytotoxic organic and organometallic bexarotene derivatives. *J. Organomet. Chem.* 2017, 839, 91–97. [CrossRef]

10. Okulova, Y.N.; Zenin, I.V.; Shutkov, I.A.; Kirsanov, K.I.; Kovaleva, O.N.; Lesovaya, E.A.; Fetisov, T.I.; Milaeva, E.R.; Nazarov, A.A. Antiproliferative activity of Pt(IV) complexes with lonidamine and bexarotene ligands attached via succinate-ethylenediamine linker. *Inorg. Chim. Acta* 2019, 495, 119010. [CrossRef]

11. Shutkov, I.A.; Antonets, A.A.; Tyurin, V.Y.; Milaeva, E.R.; Nazarov, A.A. Ruthenium(III) Complexes of NAMI-A Type with Ligands Based on Lonidamine and Bexarotene as Antiproliferative Agents. *Russ. J. Inorg. Chem.* 2021, 66, 502–509. [CrossRef]