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Mixed-ligand copper(II) complexes activate aryl hydrocarbon receptor AhR and induce CYP1A genes expression in human hepatocytes and human cell lines

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HIGHLIGHTS

- Mixed-ligand copper II complexes were studied.
- Transcriptional activity of TR, VDR, AR, GR, AhR and PXR receptors was measured.
- Target genes in primary human hepatocytes and cancer cell lines were determined.
- Two tested compounds activated AhR and induced CYP1A1 and CYP1A2.

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ABSTRACT

The effects of four copper(II) mixed-ligand complexes [Cu(qui1)(L)]$NO_3 \cdot H_2O$ (**1**–**3**) and [Cu(qui2)(phen)]NO_3 (**4**), where qui1 = 2-phenyl-3-hydroxy-4(1*H*)-quinolinone, Hqui2 = 2-(4-amino-3,5-dichlorophenyl)-*N*-propyl-3-hydroxy-4(1*H*)-quinolinone-7-carboxamide, L = 1,10-phenanthroline (phen) (**1**), 5-methyl-1,10-phenanthroline (mphen) (**2**), bathophenanthroline (bphen) (**3**), on transcriptional activities of steroid receptors, nuclear receptors and xenoreceptors have been studied. The complexes (**1**–**4**) did not influence basal or ligand-inducible activities of glucocorticoid receptor, androgen receptor, thyroid receptor, pregnane X receptor and vitamin D receptor, as revealed by gene reporter assays. The complexes **1** and **2** dose-dependently induced luciferase activity in stable gene reporter AZ-AhR cell line, and this induction was reverted by resveratrol, indicating involvement of aryl hydrocarbon receptor (AhR) in the process. The complexes **1**, **2** and **3** induced CYP1A1 mRNA in LS180 cells and CYP1A1/CYP1A2 in human hepatocytes through AhR. Electrophoretic mobility shift assay EMSA showed that the complexes **1** and **2** transformed AhR in its DNA-binding form. Collectively, we demonstrate that the complexes **1** and **2** activate AhR and induce AhR-dependent genes in human hepatocytes and cancer cell lines. In conclusion, the data presented here might be of toxicological importance, regarding the multiple roles of AhR in human physiology and pathophysiology.

1. Introduction

The use of metal-containing compounds for medicinal purposes dates back to ancient times. For instance, mercurous chloride (Hg_2Cl_2) and Arsphenamine (also known as Salvarsan or compound 606) were used for their diuretic, and antisyphilitic activities, respectively, and moreover, the latter was also used as the first modern compound in chemotherapy. An époque-making event was the introduction of the anti-cancer drug cisplatin to human chemotherapy, which was followed by other platinum-containing drugs such as oxaliplatin and carboplatin. The common

Abbreviations: AhR, aryl hydrocarbon receptor; AR, androgen receptor; CYP, cytochrome P450; DEX, dexamethasone; DHT, dihydrotestosterone; DMF, *N*,*N* dimethylformamide; GR, glucocorticoid receptor; PXR, pregnane X receptor; RIF, rifampicin; RVT, resveratrol; T3, 3,3′,5-triiodo-L-thyronine; RXRs, retinoid X receptors; TAT, tyrosine aminotransferase; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TR, thyroid hormone receptor; VD3, 25-hydroxyvitamin D3; VDR, vitamin D receptor.

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drawbacks of these anticancer drugs are their adverse and undesirable effects such as nephrotoxicity, myelosuppression and intrinsic and acquired drug-resistance. For that reason, the search for alternative metallodrugs which could suppress the above-mentioned negative effects of currently used platinum-based chemotherapeutics represents one of the challenges for bioinorganic chemists at present. Currently, a plethora of metal-based drugs were approved for human medicinal and diagnostic purposes (Dabrowiak, 2009; Gaynor and Griffith, 2012). Among them, copper(II) mixed-ligand complexes involving the amino acids and 1,10-phenanthroline-based ligands, known as Casiopeinas®, received much more attention than the others due to their remarkable cytotoxicity (Becco et al., 2014; Serment-Guerrero et al., 2011; Valencia-Cruz et al., 2013). Therefore, we have previously focused on the study of Casiopeinas®-like complexes, containing *N*-donor heterocyclic ligands, such as 1,10-phenanthroline (phen) or 2,2′-bipyridine (bpy) or their derivatives abbreviated as N-N, and 2-phenyl-3-hydroxy-4(1*H*)-quinolinone and its derivatives (H*qui*) of the general composition [Cu(N-N)(*qui*)]X·yH_2O (where X = NO_3^- or BF_4^-) (Buchtik et al., 2012; Buchtik et al., 2011; Krikavova et al., 2016; Travnicek et al., 2012). The complexes were identified as promising cytotoxic agents on a broad spectrum of human cancer cell lines showing IC_{50} values in the micromolar and sub-micromolar levels.

Many cell functions, including proliferation, differentiation, immune response, energy production and storage, detoxification or apoptosis are transcriptionally regulated by nuclear receptors, receptors for steroid hormones and xenoreceptors. Nuclear and steroid receptors are key regulators of physiological and endocrine functions, while xenoreceptors control the expression of detoxification genes. Mutual and multiple cross-talks exist between xenoreceptors and receptors for steroid hormones and nuclear receptors (Pascussi et al., 2008). Indeed, xenoreceptors aryl hydrocarbon receptor (AhR) (Diani-Moore et al., 2013; Tanos et al., 2012) and pregnane X receptor (PXR) (Moreau et al., 2008) are involved in regulation of glucose and lipid homeostasis. On the other hand, nuclear receptors and steroid receptors including vitamin D receptor (VDR) (Drocourt et al., 2002), glucocorticoid receptor (GR) (Dvorak and Pavek, 2010), androgen receptor (AR), estrogen receptor (ER) (Monostory and Dvorak, 2011), thyroid receptor (TR) and retinoic X receptor (RXR) (Pascussi et al., 2003) are important transcriptional regulators of detoxification enzymes. The interactions between drugs and ligand-activated intracellular receptors may have several implications relevant for human health, including phenomenon of endocrine disruption (Heindel et al., 2015), drug–drug interactions (Chen et al., 2014) and food-drug interactions (Margina et al., 2015).

In the current paper, we examined the effects of the complexes **1**–**4** on the transcriptional activities of AhR, AR, GR, TR, PXR and VDR, employing gene reporter assays. We found that complexes **1** and **2** activate AhR but do not influence transcriptional activities of receptors AR, GR, TR, PXR and VDR. Additionally, complexes **1** and **2** transformed AhR in its DNA-binding form and they induced AhR-dependent genes CYP1A1 and CYP1A2 in LS180 cells and in human hepatocytes. We concluded that the data presented here might be of toxicological importance, regarding the multiple roles of AhR in human physiology and pathophysiology.

2. Materials and methods

2.1. Compounds and reagents

N,*N*-Dimethylformamide (DMF), hygromycin B, dexamethasone (DEX), rifampicin (RIF), dihydrotestosterone (DHT), 3,3′,5-triiodo-L-thyronine (T3) and resveratrol (RVT) were purchased from Sigma–Aldrich (Prague, Czech Republic). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was from Ultra Scientific (Rhode Island, USA). 25-hydroxyvitamin D3 (VD3) was purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). The copper(II) complexes (**1**–**4**) with general compositions [Cu(qui1)(L)]NO_3·H_2O (**1**–**3**) and [Cu(qui2)(phen)]NO_3 (**4**) were synthesized and characterized as previously described in the literature (Buchtik et al., 2012; Buchtik et al., 2011; Travnicek et al., 2012) (Fig. 1). Luciferase lysis buffer and FuGENE® HD Transfection Reagent were obtained from Promega (Madison, WI, CA). Oligonucleotide primers used in RT-PCR reactions were synthesized by Generi Biotech (Hradec Kralove, Czech Republic). LightCycler 480 Probes Master and 480 SYBR Green I Master kit were from Roche Diagnostic Corporation (Intes Bohemia, Czech Republic). All other chemicals were of the highest quality commercially available.

2.2. Primary cultures of human hepatocytes

Human hepatocytes were isolated from human liver obtained from the multiorgan donors HH61 (M; 64 years), HH64 (M; 73 years) and HH65 (M; 34 years). Tissue acquisition protocol and the use of liver cells was approved by "Ethical committee at the Faculty Hospital Olomouc", and it was in accordance with Transplantation Act #285/2002 Coll. Cells were plated on collagen-coated dishes in hormonally and chemically defined medium (based on Ham's F12 medium and William's Medium E) as previously described (Pichard-Garcia et al., 2002) and stabilized for 24 h before the treatment. Hepatocytes were incubated in a serum-free medium for 24 h (for mRNA analyses) and 48 h (for protein analyses) with vehicle (UT; 0.1% DMF v/v), TCDD (5 nM), RIF (10 μM) and the tested complexes. Cultures were maintained at 37 °C and 5% CO_2 in a humidified incubator.

2.3. Human cancer cell lines

Human Caucasian colon adenocarcinoma cell line LS180 (ECACC No. 87021202) and human Caucasian breast adenocarcinoma cell line MCF7 (ECACC No. 86012803) were purchased from European Collection of Cell Cultures (ECACC). Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 4 mM L-glutamine, 1% non-essential amino acids, and 1 mM sodium pyruvate. Stably transfected gene reporter cell lines AZ-AHR (Novotna et al., 2011), AZ-GR (Novotna et al., 2012), AIZ-AR (Bartonkova et al., 2015) and PZ-TR (Illes et al., 2015) were described elsewhere. Cells were cultured at 37 °C and 5% CO_2 in a humidified incubator.

2.4. MTT cell viability assay

Cell lines AIZ-AR, AZ-AHR, AZ-GR, PZ-TR and LS180 were treated for 24 h with tested compounds (**1**–**4**) in concentrations ranging from 1 nM to 50 μM (unless the solubility was lower), using multi-well culture plates of 96 wells. In parallel, the cells were treated with vehicle (UT; 0.1% v/v DMF) and Triton X-100 (1%, v/v) to assess the minimal and maximal cell damage, respectively. MTT assay was performed and absorbance was measured spectrophotometrically at 570 nm on Infinite M200 (Schoeller Instruments, Prague, Czech Republic). The data were expressed as the percentage of cell viability, where 100% and 0% represent the treatments with negative control (DMF) and positive control (Triton X-100), respectively. Half-maximal inhibitory concentrations (IC_{50}) were calculated using GraphPad Prism 6 software (GraphPad Software, San Diego, USA). The tested concentrations causing the decline in viability no greater than 20% were considered as non-toxic for further experiments.