Journal of Organometallic Chemistry 964 (2022) 122300

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Microwave assisted synthesis of rhodium(I) *N*-heterocyclic carbene complexes and their cytotoxicity against tumor cell lines

Janina Schmidt, Jessica Wölker, Petra Lippmann, Ingo Ott*

Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Beethovenstr. 55, 38106 Braunschweig, Germany

ARTICLE INFO

Article history:
Received 14 January 2022
Revised 18 February 2022
Accepted 18 February 2022
Available online 22 February 2022

Keywords:
Bioorganometallic chemistry
Carbene
Cytotoxicity
Rhodium

ABSTRACT

Organometallic rhodium(I) complexes of the general type [(COD)(NHC)RhCl] (where COD = 1,5-cyclooctadiene, NHC = *N*-heterocyclic carbene) were prepared according to a two-step transmetalation method via a silver carbene intermediate. The procedure was supported by microwave irradiation allowing reduced reaction periods. The complexes were evaluated for cytotoxic effects in selected cancer cell lines, namely HT-29 colon adenocarcinoma, MDA-MB-231 breast carcinoma and MCF-7 breast adenocarcinoma, and generally triggered strong cytotoxic effects with IC_{50} values in the low micromolar range. Evaluation of structure-activity relationships clearly indicated a preference for branched isopropyl side chains at the nitrogen atoms of the NHC ligands versus non-branched ethyl side chains. Further studies on selected examples in HT-29 cells indicated that this could be the result of a higher cellular uptake.

1. Introduction

Cisplatin and other square-planar platinum species have been playing a major role in clinical cancer chemotherapy over many decades and have shaped the field of inorganic medicinal chemistry. Motivated by the tremendous success of the platinum anticancer drugs, a considerable number of different metal-based potential anticancer agents has been designed and studied for application in tumor therapy, however, without reaching the success of the lead compound cisplatin [1]. Interestingly, among the numerous investigated metallodrug candidates there are comparably few non-platinum examples that exhibit a cisplatin-like square-planar geometry at the metal center.

We and others have recently reported on the antitumor potential of square-planar organometallic rhodium(I) complexes of the type [(COD)(NHC)RhX] (where COD = 1,5-cyclooctadiene, NHC = *N*-heterocyclic carbene and X = halide, see Fig. 1 for some selected examples) [2–14].

Rhodium complexes in general have been less frequently studied as anticancer drugs and the majority of the recently investigated types are Rh(III) complexes [15–25].

If compared with other metal complexes, the number of reports on rhodium(I) anticancer agents is still relatively low, although the antitumor potential of square-planar organometallic Rh(I) complexes with COD ligands had been described soon after the discovery of the antitumor activity of cisplatin [26,27]. Rh(I)-based polymeric nanomicelles have also demonstrated in-vivo anticancer activity [28]. In particular very few studies on [(COD)(NHC)RhX] anticancer complexes are available, however, with very promising results [2–14].

Many of the investigated complexes show appreciable cytotoxicity against cultured tumor cell lines and the overcoming of drug resistance in leukemia cells was reported for a selected complex [2]. Studies on the effects on the cellular signalling in cancer cell lines revealed strong phosphorylation (activation) of several relevant proteins, including the heat show protein 27 (HSP27) and mitogen-activated protein kinases (MAPKs), such as p38 and ERK1/2, indicative of a general cellular stress response [3]. Importantly, such biological response pattern was different from that observed with cisplatin. An other example showed good antitumor activity in-vivo [12].

Based on the square-planar geometry and the fact that Rh(I) is isoelectronic with Pt(II), the DNA has been considered as a relevant molecular target for [(COD)(NHC)RhX] complexes. In fact, one complex was shown to halt DNA replication and alter cell migration [4]. An other example showed a higher amount of binding to the DNA than cisplatin in precipitation experiments with isolated DNA, however, cellular uptake experiments indicated that only a very small percentage of the complex reached the nuclei of cancer cells [2]. For complexes that contained a DNA intercalating naphthalimide structure in the NHC ligand, both intercalative interaction with the DNA (related to the naphthalimide moiety) and co-

* Corresponding author.
E-mail address: ingo.ott@tu-bs.de (I. Ott).

https://doi.org/10.1016/j.jorganchem.2022.122300
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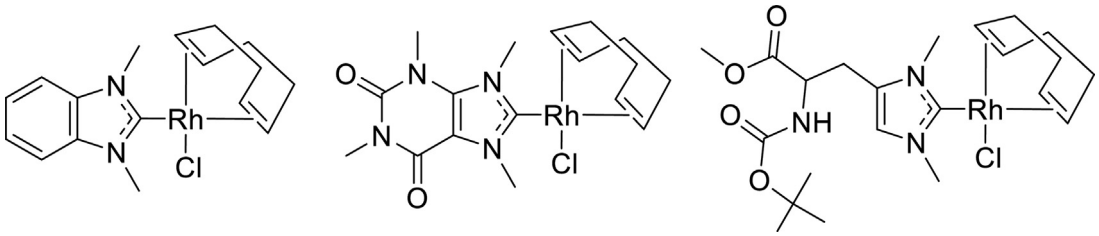


Fig. 1. Examples of previously studied [(COD)(NHC)RhCl] complexes [2,9,10].

ordinative binding (under loss of the chlorido ligand) to the model nucleoside base 9-ethylguanine were confirmed [7,8].

Besides DNA, also protein targets have been considered for [(COD)(NHC)RhX] complexes [29]. Protein crystallography studies of selected complexes with hen-egg-white lysozyme as a model protein showed major adducts at a histidine residue. Interestingly, oxidation of Rh(I) to Rh(III) was noted changing the geometry of the rhodium center from square-planar to octahedral [10]. Binding to histidine was also noted for a Rh(III) complex with a NHC and a cyclopentadienyl ligand [30]. In this case the NHC ligand was replaced upon binding to the protein. For similar iridium complexes of the type [Ir(NHC)(COD)Cl] a ligand exchange mechanism was proposed, which consisted of an initial loss of the COD and chloride ligands and, depending on the conditions, further reaction of the remaining fragment with biomolecules or decomposition [31]. Notably, oxidation of the metal upon interaction with proteins has also been confirmed for such iridium complexes and they generally displayed very promising in-vitro anticancer activity [32–34].

Moderate inhibition of the selenoenzyme thioredoxin reductase (TrxR) was reported for some examples of [(COD)(NHC)RhX] complexes [2,9]. Such TrxR inhibition might indirectly contribute to the anticancer mechanism as the resulting upregulation of reactive oxygene species formation contributes to DNA damage [9].

[(COD)(NHC)RhX] complexes also showed good activity against the SARS-CoV-2 papain-like protease PL^{pro}, however, due to their too strong cytotoxicity against host cells, they did not proceed to further antiviral studies [35].

Regarding the cellular localisation, besides the expected mitochondrial uptake, accumulation in the endoplasmic reticulum (ER) was reported by Metzler-Nolte et al. [11] calling for further investigation of possible induction of ER stress by the complexes.

Finally, the potential of [(COD)(NHC)RhX] complexes as bioorganometallic catalysts, e.g. for hydrosilylation, should be mentioned [36–38].

Taken together, the ongoing studies on the mechanisms of [(COD)(NHC)RhX] complexes indicate that such complexes have multitarget anticancer properties that can be fine-tuned by careful optimization of the coordinated ligands, in particular the NHC partial structures. Further studies involving different NHC core structures and elucidation of structure-activity-relationships should therefore have a high priority in the further development of the complexes.

In the here reported work we introduced phenylimidazole derived NHC ligand structures, for which we had reported very promising results as ligands of bioactive gold complexes in recent studies [39], into complexes of the general type [(COD)(NHC)RhCl] aiming at establishing more structure-activity-relationships regarding the NHC ligands. Thus, the influence of different halide substituents on the para-position of the phenyl ring as well as the side chains (non-branched: ethyl, branched: isopropyl) on the NHC nitrogen atoms were evaluated. The resulting eight target complexes were investigated for cytotoxic effects in a panel of cancer lines. In addition to that, we have evaluated the application of microwave technology in the synthesis of the target complexes.

2. Experimental

2.1. General

Chemicals and reagents were obtained from Sigma–Aldrich, TCI, Alfa Aesar and ACROS unless otherwise noted. NMR spectra were recorded on a Bruker DRX-400 AS or an AV III HD 500 NMR spectrometer. Positive-ion ESI (electrospray ionization) mass spectra were recorded on a Finnigan MAT95 XL. Elemental analyses were conducted in a Flash EA1112 apparatus. A VictorTM X4 Perkin–Elmer 2030 multilabel reader was used for the biological assays. Cell culture: MCF-7, MDA-MB-231, HT-29, Caco-2 and Calu-3 cells were obtained from DSMZ (Braunschweig) and were maintained in Dulbecco's modified Eagle's medium (DMEM; 4.5 g/L D-glucose, l-glutamine, pyruvate), supplemented with fetal bovine serum superior, standardized (Bio&Sell, 10% v/v) and gentamycin (50 mg/L) with a weekly passage.

2.2. General synthesis of the phenylimidazolium halides (**2a–2h**)

An amount of 0.20 g of the respective phenylimidazole **1a** - **1d** and 1.0 equivalent of K_2CO_3 were added to 2.0 mL of acetonitrile, 3 equivalents of ethyl iodiode (for **2a** – **2d**) or 2-bromopropane (for **2e** – **2h**), respectively, were added, and the mixture was reacted for 8-54 h in a microwave at 82°C, 50 W and 10 psi. The progress of the reaction was monitored by thin layer chromatography. The liquid phase was removed, the solid phase washed with methanol, and the volume of the combined liquid phases was reduced using a rotary evaporator. Further work-up for **2a** – **2d**: The residue was dissolved in dichloromethane and filtered using a syringe filter (0.2 µM) to remove the remaining K_2CO_3. Dichloromethane was removed by evaporation under reduced pressure resulting in an oily residue with some precipitate. A small amount of acetonitrile was added for dissolution and the product was precipitated with 10 mL of 1/1 ethylacetate/*n*-hexane (V/V) and repeatedly cooled for 24 h at -25°C until no further precipitate was obtained. The combined precipitates were dried using a rotary evaporator followed by drying under reduced pressure at 40°C for a period of 3 days. Further work-up for **2e** – **2h**: The products were purified by column chromatography over silica with ethylacetate/*n*-hexane/methanol mixtures (8/2/0 → 8/2/1 → 8/2/10) as eluent. The volume of the combined eluates was reduced with a rotary evaporator, filtered using a syringe filter (0.2 µm), and dried under reduced pressure at 40°C for 3 days.

1,3-Diethyl-(4-phenyl)-1*H*-imidazol-3-iumiodid (**2a**) was already described in the literature [39].

1,3-Diethyl-4-(4'-chlorophenyl)-1*H*-imidazol-3-iumiodid (**2b**) was already described in the literature [39].

1,3-Diethyl-4-(4'-chlorophenyl)-1*H*-imidazol-3-iumiodide (**2c**) general method with **1c**, reaction period: 28 h, precipitate with EtOAc, brown solid, yield: 35%

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.33 (d, $^4J_{H,H}$ = 1.8 Hz, 1H, CH, C2), 7.51 (m, 2H, 2CH, C6' + C2'), 7.42 (m, 2H, 2CH, C3' + C5'), 7.37 (d, $^3J_{H,H}$ = 1.8 Hz, 1H, CH, C5), 4.50 (q, $^3J_{H,H}$ = 7.4 Hz, 2H, CH_2,

C8), 4.29 (q, $^3J_{H,H}$ = 7.4 Hz, 2H, CH_2, C6), 1.66 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3, C9), 1.51 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, CH_3, C7) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.34 (s, CH, C2), 136.77 (s, C_q, C4'), 134.09 (s, C_q, C4) 130.98, (s, C_q, C1'), 129.87 (s, 2CH, C5' + C3'), 123.28 (s, 2CH, C2' + C6'), 119.37 (s, CH, C5), 45.72 (s, CH_2, C6), 43.49 (s, CH_2, C8), 15.76 (d, $^3J_{C,C}$ = 21.97 Hz, $2CH_3$, C7 + C9) ppm; elemental analysis: $C_{13}H_{16}ClIN_2$ (calc./found [%]): C (43.06 / 43.02), H (4.45 / 4.60), N (7.73 / 7.56); MS (EI): m/z =235.10 $[M\text{-}Br]^+$

1,3-Diethyl-4-(4'-bromophenyl)-1*H*-imidazol-3-iumiodide (**2d**) was already described in the literature [39]

1,3-Diisopropyl-(4-phenyl)-1*H*-imidazol-3-iumbromid (**2e**)
general method with **1a**, reaction period: 54 h, eluent for column chromatography: EtOAc:*n*-hexane:MeOH (8/2/1 → 8/2/100), yellow powder, yield: 11%

^{1}H NMR (400 MHz, $CDCl_3$): δ 11.05 (d, $^4J_{H,H}$ = 1.7 Hz, 1H, C2), 7.61 – 7.52 (m, 3H, C4' + C3' + C5'), 7.41 – 7.38 (m, 2H, C2' + C6'), 7.23 (d, $^4J_{H,H}$ = 1.4 Hz, 1H, C5), 5.24 (hept, $^3J_{H,H}$=6.7 Hz, 1H, C9), 4.52 (hept, $^3J_{H,H}$ = 6.7 Hz, 1H, C6), 1.69 (d, $^3J_{H,H}$ = 6.7 Hz, 12H, C11 + C10) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.10 (s, CH, C2), 134.89 (s, C_q, C4), 130.86 (s, CH, C4'), 129.64 (d, $^3J_{c,c}$ = 23,55 Hz, 4 CH, C6' + C2' + C3' + C5'), 125.24 (s, C_q, C1'), 116.12 (s, CH, C5), 53.47 (s, CH, C6), 51.44 (s, CH, C9), 23.63 (d, $^2J_{c,c}$ = 23,45 Hz, 4 CH_3, C7 + C8 + C10 + C11) ppm; elemental analysis: $C_{15}H_{21}BrN_2$ (calc./found [%]): C (58.26 / 58.59), H (6.94 / 6.84), N (8.73 / 9.06); MS (EI): m/z =219.13 $[M\text{-}Br+H]^+$

1,3-Diisopropyl-4-(4'-fluorophenyl)-1*H*-imidazol-3-iumbromid (**2f**)
general method with **1b**, reaction period: 38 h, white powder, yield: 34%

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (d, $^4J_{H,H}$ = 1.8 Hz, 1H, C2), 8.09 (d, $^4J_{H,H}$ = 1.8 Hz, 1H, C5), 7.64 (m, 2H, C2' + C6'), 7.45 (m, 2H, C3' + C5'), 4.69 (hept, $^3J_{H,H}$ = 6.8 Hz, 1H, C9), 4.48 (hept, $^3J_{H,H}$ = 6.8 Hz, 1H, C6), 1.55 (d, $^3J_{H,H}$ = 6.7 Hz, 6H, C10 + C11), 1.45 (d, $^3J_{H,H}$ = 6.7 Hz, 6H, C7 + C8) ppm; ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.01 (d, $^1J_{C,F}$ = 248.1 Hz, C_q, C4'), 133,43 (s, CH, C2), 132.75 (s, C_q, C4), 132.27 (d, $^3J_{C,F}$ = 8.8 Hz, 2CH, C2' + C6'), 122.13 (d, $^4J_{C,F}$ = 3.2 Hz, C_q, C1'), 118.70 (s, CH, C5), 116.26 (d, $^3J_{C,F}$ = 22.0 Hz, 2CH, C3' + C5'), 52.67 (s, CH, C6), 50.08 (s, CH, C9), 39.42 (dp, $^2J_{C,C}$ = 42.0, $^3J_{C,C}$ = 21.0 Hz, $4CH_3$, C7 + C8 + C10 + C11) ppm; ^{19}F NMR (471 MHz, DMSO-d_6) δ -110.06 (tt, $^3J_{F,H}$ = 8.9 Hz, $^4J_{F,H}$ = 5.4 Hz) ppm; elemental analysis: $C_{15}H_{20}BrFN_2$ (calc./found [%]): C (55.06 / 55.69), H (6.16 / 6.23), N (8.56 / 8.64); MS (EI): m/z =246.18 $[M\text{-}Br+H]^+$

1,3-Diisopropyl-4-(4'-chlorophenyl)-1*H*-imidazol-3-iumiodide (**2g**)
general method with **1c**, reaction period: 25 h, white powder, yield: 39%

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.02 (s, 1H, CH, C2), 7.54 (d, $^4J_{H,H}$ = 7.9 Hz, 2H, 2CH, C2' + C6'), 7.37 (d, $^4J_{H,H}$ = 8.1 Hz, 2H, 2CH, C3' + C5'), 7.32 (s, 1H, CH, C5), 5.23 (hept, $^3J_{H,H}$ = 6.7 Hz, 1H, CH, C9), 4.49 (hept, $^3J_{H,H}$ = 6.7 Hz, 1H, CH, C6), 1.68 (dd, $^3J_{H,H}$ = 6.3 Hz, 12H, $4CH_3$, C7 + C8 + C10 + C11) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.34 (s, CH, C2), 136.19 (s, C_q, C4'), 133.64 (s, C_q, C4) 131.12 (s, 2CH, C3' + C5'), 129.88 (s, 2CH, C2' + C6'), 123.66 (s, C_q, C1'), 116.69 (s, CH, C5), 53.60 (s, CH, C6), 51.53 (s, CH, C9), 23.68 (s, $2CH_3$, C7 + C8), 23.42 (s, $2CH_3$, C10 + C11) ppm; elemental analysis: $C_{15}H_{20}BrClN_2$ (calc./found [%]): C (52.42 / 51.64), H (5.87 / 5.81), N (8.15 / 7.83); MS (EI): m/z =164.65 $[M\text{-}Br]^+$

1,3-Diisopropyl-4-(4'-bromophenyl)-1*H*-imidazol-3-iumbromide (**2h**)
general method with **1d**, reaction period: 39 h, white powder, yield: 66%

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.70 (s, 1H, CH, C2), 7.81 (m, 2H, 2CH, C2' + C6'), 7.69 (d, $^4J_{H,H}$ = 8.2 Hz, 1H, CH, C5), 7.58 (m, 2H, 2CH, C3' + C5'), 4.95 (hept, $^3J_{H,H}$ = 6.7 Hz, 1H, CH, C9), 4.49 (hept, $^3J_{H,H}$ = 6.7 Hz, 1H, CH, C6), 1.67 (m, 12H, $4CH_3$, C7 + C8 + C10 + C11) ppm; ^{13}C NMR (121 MHz, $CDCl_3$) δ 135.65 (s, CH, C2), 134.01 (d, $^1J_{Br,C}$ = 3.22 Hz, C_q, C4'), 133.88 (s, C_q, C4) 132.86 (s, 2CH, C3' + C5'), 132.63 (s, 2CH, C2' + C6'), 124.06 (s, C_q, C1'), 116.82 (s, CH, C5), 53.75 (s, CH, C6), 51.56 (s, CH, C9), 23.60 (s, $2CH_3$, C7 + C8), 23.34 (s, $2CH_3$, C10 + C11) ppm; elemental analysis: $C_{15}H_{20}Br_2N_2$ (calc./found [%]): C (46.42 / 39.99), H (5.19 / 4.28), N (7.22 / 7.54); MS (EI): m/z = 307.08 $[M\text{-}Br]^+$

2.3. General synthesis of (COD)(NHC)RhCl complexes (*3a–3h*)

An amount of 0.10 g (2 equivalents) of the respective phenylimidazolium precursor (**2a** – **2h**) was dissolved in 2.0 mL dichloromethane, 1 equivalent of silver(I)oxide were added and the mixture was stirred for 15 min at room temperature. For transmetalation 1 equivalent of $[Rh(COD)Cl]_2$ was added and reacted for 3-5 h (see below) at 25°C, 10 psi and 50 W in a microwave apparatus (CEM). The formed silver halide was removed by filtration and the product was purified chromatography over silica with dichloromethane / methanol mixtures as eluent (1/0 → 1/0.5). The volume of the combined eluted pure fractions was reduced using a rotary evaporator, filtered using a 0.2 µm syringe filter, treated with *n*-hexane, and dried for 3 days at 40°C under reduced pressure to yield the crystalline product.

Chlorido[1,3-diethyl-(4-phenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3a**)
general method with **2a**; reaction period: 3 h, yellow solid, yield: 87%. Complex **3a** was already reported in the literature [35].

Chlorido[1,3-diethyl-4-(4'-fluorophenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3b**)
general method with **2b**, reaction period: 4 h, yellow powder, yield: 77%. Complex **3b** was already reported in the literature [35].

Chlorido[1,3-diethyl-4-(4'-chlorophenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3c**)
general method with **2c**, reaction period: 5 h; yellow powder; yield: 46%

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.39 (m, 2H, 2CH, C2' + C6'), 7.28 – 7.24 (m, 2H, 2 CH, C3' + C5'), 6.81 (s, 1H, CH, C5), 5.09 – 4.99 (m, 2H, 2 CH, 2 *trans*-CH-COD), 4.85 (dq, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 7.3 Hz, 1H, CH_2, C8), 4.72 (dq, $^2J_{H,H}$ = 13.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 1H, CH_2, C6), 4.58 (dq, $^2J_{H,H}$ = 14.67 Hz, $^3J_{H,H}$ = 7.29 Hz, 1H, CH_2, C6), 4.50 (dq, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 7.1 Hz, 1H, CH_2, C8), 3.36 (m, 2H, 2 CH, 2 *cis*-CH-COD), 2.52 – 2.33 (m, 4H, 2 CH_2 axial, COD), 1.96 (m, 4H, 2 CH_2 equatorial, COD), 1.56 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, CH_3, C9), 1.27 (t, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3, C7) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 183.42 (d, $^1J_{Rh,C}$ = 51.02 Hz, C_q, C2), 134.97 (s, C_q, C4'), 133.54 (s, C_q, C1'), 130.02 (s, 2 CH, C2' + C6'), 129.12 (s, 2 CH, C3' + C5'), 127.57 (s, C_q, C4'), 118.45 (s, CH, C5), 98.45 (dd, $^1J_{Rh,C}$ = 6.84 Hz, $^2J_{Rh,C}$ = 4.13 Hz, 2 CH, 2 *trans*-CH-COD), 68.33 (d, $^1J_{Rh,C}$ = 14.94 Hz, CH, *cis*-CH-COD), 67.99 (d, $^1J_{Rh,C}$ = 14.86 Hz, CH, *cis*-CH-COD), 45.90 (s, CH_2, COD), 43.92 (s, CH_2, COD), 33.13 (s, CH_2, COD), 32.77 (s, CH_2, COD), 29.04 (s, CH_2, C6), 28.72 (s, CH_2, C8), 16.33 (s, CH_3, C7), 16.19 (s, CH_3, C9) ppm; elemental analysis: $C_{21}H_{27}Cl_2N_2Rh$ (calc. / found [%]): C (52.41 / 52.90), H (5.66 / 5.74), N (5.82 / 5.54); MS (EI): m/z = 479.9 $[M]^+$, 444.0 $[M\text{-}Cl]^+$

Chlorido[1,3-diethyl-4-(4'-bromophenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3d**)
general method with **2d**, reaction period: 5 h; yellow powder, yield: 65%

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.58 – 7.55 (m, 2H, 2 CH, C2' + C6'), 7.22 – 7.19 (m, 2H, 2 CH, C3' + C5'), 6.81 (s, 1H, CH, C5), 5.08 – 5.00 (m, 2H, 2 CH, 2 *trans*-CH-COD), 4.84 (dq, $^2J_{H,H}$ = 14.8 Hz, $^3J_{H,H}$ = 6.78 Hz, 1H, CH_2, C8), 4.71 (dq, $^2J_{H,H}$ = 13.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 1H, CH_2, C6), 4.58 (dq, $^2J_{H,H}$ = 14.8 Hz, $^3J_{H,H}$ = 6.78 Hz, 1H, CH_2, C6), 4.51 (dq, $^2J_{H,H}$ = 14.11 Hz, $^3J_{H,H}$ = 7.23 Hz, 1H, CH_2, C8), 3.41 – 3.30 (m, 2H, 2 CH, 2 *cis*-CH-COD), 2.50 – 2.33 (m, 4H,

2 CH_2 axial, COD), 2.04 – 1.89 (m, 4H, 2 CH_2 equatorial, COD), 1.55 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, CH_3, C9), 1.27 (t, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3, C7) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 183.46 (d, $^1J_{Rh,C}$ = 51.09 Hz, C_q, C2), 133.54 (s, C_q, C4), 132.08 (s, 2 CH, C2' + C6'), 130.24 (s, 2 CH, C3' + C5'), 128.02 (s, C_q, C1'), 123.11 (s, C_q, C4'), 118.42 (s, CH, C5), 98.45 (dd, $^1J_{Rh,C}$ = 6.9 Hz, $^2J_{Rh,C}$ = 5.0 Hz, 2 CH, 2 *trans*-CH-COD), 68.33 (d, $^1J_{Rh,C}$ = 14.6 Hz, CH, *cis*-CH-COD), 68.01 (d, $^1J_{Rh,C}$ = 14.6 Hz, CH, *cis*-CH-COD), 45.89 (s, CH_2, COD), 43.93 (s, CH_2, COD), 33.12 (s, CH_2, COD), 32.76 (s, CH_2, COD), 29.03 (s, CH_2, C6), 28.72 (s, CH_2, C8), 16.33 (s, CH_3, C7), 16.19 (s, CH_3, C9) ppm; elemental analysis: $C_{21}H_{27}BrClN_2Rh$ (calc. / found [%]): C (47.98 / 49.02), H (5.18 / 5.43), N (5.33 / 5.14); MS (EI): m/z = 524.0 $[M]^+$, 487.9 $[M\text{-}Cl]^+$.

Chlorido[1,3-diisopropyl-(4-phenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3e**)

general method with **2e**; reaction period: 5 h; yellow powder, yield: 57%

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.44 – 7.36 (m, 3H, 3 CH, C3' + C4' + C5'), 7.34 – 7.31 (m, 2H, 2 CH, C2' + C6'), 6.71 (s, 1H, C5), 5.95 (h, $^3J_{H,H}$ = 7.1 Hz, 1H, CH, C9), 5.88 (h, $^3J_{H,H}$ = 6.8 Hz, 1H, CH, C6), 5.04 – 4.97 (m, 2H, 2 CH, 2 *trans*-CH-COD), 3.46 – 3.40 (m, 2H, 2 CH, 2 *cis*-CH-COD), 2.53 – 2.33 (m, 4H, 2 CH_2 axial, COD), 2.01 – 1.89 (m, 4H, 2 CH_2 equatorial, COD), 1.52 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, 2 CH_3, C10 + C11), 1.43 (dd, $^3J_{H,H}$ = 8.1 Hz, $^4J_{H,H}$ = 7.0 Hz, 6H, 2 CH_3, C7 + C8) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 180.54 (d, $^1J_{Rh,C}$ = 50.6 Hz, C_q, C2), 134.28 (s, C_q, C4), 131.40 (s, CH, C4'), 130.34 (s, CH, phenyl- CH), 129.04 (s, CH, phenyl- CH), 127.98 (s, CH, phenyl-CH), 116.47 (s, CH, phenyl- CH), 97.55 (dd, $^1J_{Rh,C}$ = 14.82 Hz, $^2J_{Rh,C}$ = 7.03 Hz, 2 *trans*-CH-COD), 67.59 (dd, $^1J_{Rh,C}$ = 14.84 Hz, $^2J_{Rh,C}$ = 7.07 Hz, 2 *cis*-CH-COD), 54.58 (s, CH, C6), 52.70 (s, CH, C9), 33.06 (s, CH_2, COD), 32.74 (s, CH_2, COD), 28.98 (s, CH_2, COD), 28.75 (s, CH_2, COD), 24.23 (s, CH_3, C7), 23.61 (s, CH_3, C8), 23.35 (s, CH_3, C10), 23.22 (s, CH_3, C11) ppm; elemental analysis: $C_{23}H_{32}ClN_2Rh$ (calc./found [%]): C (58.17 / 58.08), H (6.79 / 6.88), N (5.90 / 5.1); MS (EI): m/z = 474.13 $[M]^+$, 438.1 $[M\text{-}Cl]^+$.

Chlorido[1,3-diisopropyl-4-(4'-fluorophenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3f**)

general method with **2f**, reaction period: 5 h, eluent for column chromatography: dichloromethane; yellow powder, yield: 89%

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.34 – 7.27 (m, 2H, 2 CH, C3' + C5'), 7.12 – 7.05 (m, 2H, 2 CH, C2' + C6'), 6.71 (s, 1H, CH, C5), 5.97 (hept, $^3J_{H,H}$ = 7.0 Hz, 1H, CH, C9), 5.88 (hept, $^3J_{H,H}$ = 6.8 Hz, 1H, CH, C6), 5.01 (m, 2H, 2 CH, 2 *trans*-CH-COD), 3.50 – 3.38 (m, 2H, 2 CH, 2 *cis*-CH-COD), 2.50 – 2.33 (m, 4H, 2 CH_2 axial, COD), 2.02 – 1.87 (m, 4H, 2 CH_2 equatorial, COD), 1.52 (dd, $^3J_{H,H}$ = 6.8 Hz, $^4J_{H,H}$ = 1.1 Hz, 6H, 2 CH_3, C10 + C11), 1.41 (dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 7.0 Hz, 6H, 2 CH_3, C7 + C8) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 180.96 (d, $^1J_{Rh,C}$ = 50.89 Hz, Cq, C2), 163.12 (d, $^1J_{C,F}$ = 248.89 Hz, Cq, C4') 133.30 (d, $^4J_{C,F}$ = 8.35 Hz, 2 CH, C2' + C6'), 133.02 (s, Cq, C4), 126.24 (d, $^4J_{C,F}$ = 3.6 Hz, Cq, C1'), 116.81 (s, CH, C5), 115.11 (d, $^3J_{C,F}$ = 23,79 Hz, 2 CH, C3' + C5'), 97.69 (dd, $^1J_{Rh,C}$ = 16.25 Hz, $^2J_{Rh,C}$ = 7.47 Hz, 2 *trans*-CH-COD), 67.60 (dd, $^1J_{Rh,C}$ = 16.30 Hz, $^2J_{Rh,C}$ = 7.33 Hz, 2 *cis*-CH-COD), 54.62 (s, CH, C6), 52.73 (s, CH, C9), 33.01 (s, CH_2, COD), 32.78 (s, CH_2, COD), 28.93 (s, CH_2, COD), 28.78 (s, CH_2, COD), 24.21 (s, CH_3, C8), 23.59 (s, CH_3, C7), 23.36 (s, CH_3, C10), 23.22 (s, CH_3, C11) ppm; ^{19}F NMR (471 MHz, $CDCl_3$) δ -112.19 (tt, $^3J_{F,H}$ = 8.6 Hz, $^4J_{F,H}$ = 5.3 Hz, ^{1}F) ppm; elemental analysis: $C_{23}H_{31}ClFN_2Rh$ (calc. / found [%]): C (56.05 / 56.03), H (6.34 / 6.31), N (5.68 / 5.63); MS (EI): m/z = 492.0 $[M]^+$, 456.0 $[M\text{-}Cl]^+$

Chlorido[1,3-diisopropyl-4-(4'-chlorophenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3g**)

general method with **2g**, reaction period: 5 h, yellow powder; yield: 42%

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39 – 7.36 (m, 2H, 2 CH, C3' + C5'), 7.28 – 7.24 (m, 2H, 2 CH, C2' + C6'), 6.71 (s, 1H, CH, C5), 5.96 (hept, $^3J_{H,H}$ = 7.0 Hz, 1H, CH, C9), 5.88 (hept, $^3J_{H,H}$ = 7.1 Hz, 1H, CH, C6), 5.04 – 4.98 (m, 2H, 2 CH, 2 *trans*-CH-COD), 3.45 – 3.38 (m, 2H, 2 CH, 2 *cis*-CH-COD), 2.49 – 2.33 (m, 4H, 2 CH_2 axial, COD), 2.01 – 1.90 (m, 4H, 2 CH_2 equatorial, COD), 1.52 (dd, $^3J_{H,H}$ = 6.8 Hz, $^4J_{H,H}$ = 0.8 Hz, 6H, 2 CH_3, C10 + C11), 1.42 (dd, $^3J_{H,H}$ = 7.1 Hz, $^4J_{H,H}$ = 4.3 Hz, 6H, 2CH_3, C7 + C8) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ = 181.45 (d, $^1J_{Rh,C}$ = 50.64 Hz, C_q, C2), 135.34 (s, C_q, C4), 132.94 (s, C_q, C4'), 132.68 (s, 2 CH, C2' + C6'), 128.76 (s, C_q, C1'), 128.32 (s, 2 CH, C3' + C5'), 116.78 (s, CH, C5), 97.73 (dd, $^1J_{Rh,C}$ = 14.97 Hz, $^2J_{Rh,C}$ = 7.46 Hz, 2 *trans*-CH-COD), 64.64 (dd, $^1J_{Rh,C}$ = 15.21 Hz, $^2J_{Rh,C}$ = 7.53 Hz, 2 *cis*-CH-COD) 54.64 (s, CH, C6), 52.78 (s, CH, C9), 33.02 (s, CH_2, COD), 32.76 (s, CH_2, COD), 28.94 (s, CH_2, COD), 28.76 (s, CH_2, COD), 24.21 (s, CH_3, C7), 23.65 (s, CH_3, C8), 23.38 (s, CH_3, C10), 23.22 (s, CH_3,C11) ppm; elemental analysis: $C_{23}H_{31}Cl_2N_2Rh$ (calc. / found [%]): C (54.24 / 53.87), H (6.14 / 6.35), N (5.50 / 5.10); MS (EI): m/z = 508.1 $[M]^+$, 472.7 $[M\text{-}Cl]^+$

Chlorido[1,3-diisopropyl-4-(4'-chlorophenyl)-imidazol-2-ylidene(cycloocta-1,5-diene)]rhodium(I) (**3h**)

general method with 2h, reaction period: 5 h, eluent for column chromatography: EtOAc:*n*- hexane:MeOH (8/ 2/0 → 8/2/10), yellow powder, yield: 42%

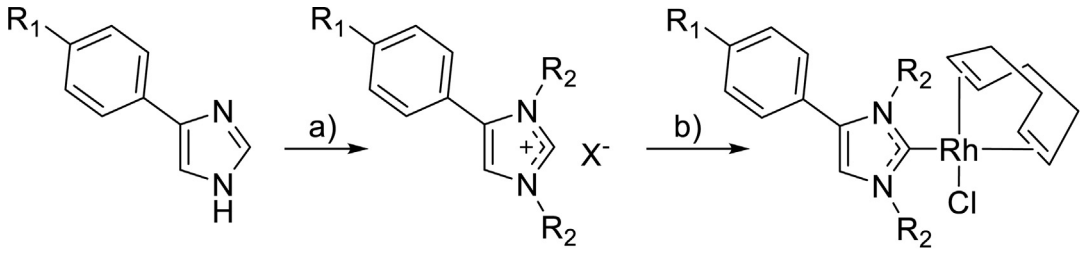
^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.55 – 7.51 (m, 2H, 2 CH, C3' + C5'), 7.21 – 7.18 (m, 2H, 2 CH, C2' + C6'), 6.71 (s, 1H, CH, C5), 5.96 (hept, $^3J_{H,H}$ = 7.0 Hz, 1H, CH, C9), 5.88 (hept, $^3J_{H,H}$ = 6.8 Hz, 1H, CH, C6), 5.05 – 4.96 (m, 2H, 2 CH, 2 *trans*-CH-COD), 3.44 – 3.37 (m, 2H, 2 CH, 2 *cis*-CH-COD), 2.54 – 2.32 (m, 4H, 2 CH_2 axial, COD), 2.02 – 1.88 (m, 4H, 2 CH_2 equatorial, COD), 1.52 (dd, $^3J_{H,H}$ = 6.84 Hz, $^4J_{H,H}$ = 1.1 Hz, 6H, 2 CH_3, C10 + C11), 1.42 (dd, $^3J_{H,H}$ = 7.0 Hz, $^3J_{H,H}$ = 4.2 Hz, 6H, 2 CH_3, C7 + C8) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ = 181.29 (d, $^1J_{Rh,C}$ = 51.46 Hz, C_q, C2), 132.96 (s, C_q, C4) 132.93 (s, 2 CH, C2' + C6'), 131.29 (s, C3' + C5'), 129.24 (s, C_q, C4'), 123.54 (s, C_q, C1') 116.73 (s, CH, C5), 97.74 (dd, $^1J_{Rh,C}$ = 14.85 Hz, $^2J_{Rh,C}$ = 6,94 Hz, 2 CH, 2 *trans*-CH-COD), 67.64 (dd, $^1J_{Rh,C}$ = 15,10 Hz, 2 CH, 2 *cis*-CH-COD), 54.65 (s, CH, C6), 52.79 (s, CH, C9), 33.02 (s, CH_2, COD), 32.75 (s, CH_2, COD), 28.94 (s, CH_2, COD), 28.76 (s, CH_2, COD), 24,21 (s, CH_3, C7), 23.66 (s, CH_3, C8), 23.39 (s, CH_3, C10), 23.22 (s, CH_3, C11) ppm; elemental analysis: $C_{23}H_{31}BrClN_2Rh$ (calc. / found [%]): C (49.89 / 50.38), H (5.64 / 5.88), N (5.06 / 5.1); MS (EI): m/z = 553.9 $[M]^+$, 518.9 $[M\text{-}Cl]^+$

2.4. Antiproliferative effects in tumor cells

The antiproliferative effects were determined according to standard protocols. In short: A volume of 100 µL of HT-29 cells (2,565 cells/mL), MDA-MB-231 cells (4,120 cells/mL) and MCF-7 cells (4,840 cells/mL) was transferred into the wells of a 96-well plates and incubated at 37°C under 5% CO_2 for 72 h. Stock solutions of the compounds were freshly prepared in dimethylformamide (DMF) and diluted with the cell culture medium to obtain various concentrations (final concentration of DMF: 0.1 % v/v). After 72 h (HT-29) or 96 h (MDA-MB-231, MCF-7) of exposure, the biomass of the cells was determined via crystal violet staining and the IC_{50} value was determined as the concentration that caused 50% inhibition of cell proliferation relative to an untreated control. The results were calculated as the mean values of three independent experiments.

2.5. Toxic effects against cell layers with high confluency

Caco-2 and Calu-3 cells were grown as almost confluent monolayers in 96-well plates. Complex **3f** was dissolved as stock solution in DMF and diluted with cell culture medium to graded concentrations. The cell layers were incubated with the drug containing media for 24 h at 37°C / 5% CO_2 in an incubator. The cell viability was determined by crystal violet staining and cell viability was calcu-



1a-1d **2a - 2h** **3a - 3h**

2a: R_1 = H, R_2 = ethyl, X = I
2b: R_1 = F, R_2 = ethyl, X = I
2c: R_1 = Cl, R_2 = ethyl, X = I
2d: R_1 = Br, R_2 = ethyl, X = I
2e: R_1 = H, R_2 = isopropyl, X = Br
2f: R_1 = F, R_2 = isopropyl, X = Br
2g: R_1 = Cl, R_2 = isopropyl, X = Br
2h: R_1 = Br, R_2 = isopropyl, X = Br

3a: R_1 = H, R_2 = ethyl
3b: R_1 = F, R_2 = ethyl
3c: R_1 = Cl, R_2 = ethyl
3d: R_1 = Br, R_2 = ethyl
3e: R_1 = H, R_2 = isopropyl
3f: R_1 = F, R_2 = isopropyl
3g: R_1 = Cl, R_2 = isopropyl
3h: R_1 = Br, R_2 = isopropyl

Scheme 1. Synthesis of [(COD)(NHC)RhCl] complexes a) ethyl iodide or 2-bromopropane, K_2CO_3 (82°C, 10 psi, 50 W), b) Ag_2O (r.t.), $[Rh(COD)Cl]_2$ (25°C, 10 psi, 50 W)

lated as percentage of an untreated control. Results were obtained in three independent experiments.

2.6. Cellular uptake

For cellular-uptake studies HT-29 cells were grown until at least 70% confluency in 75 cm^2 cell culture flasks. Stock solutions of complex **3b** or **3f** in DMF were freshly prepared and diluted with cell culture medium to the desired concentration of 2.0 µM (final DMF concentration: 0.1% v/v). The cell culture medium of the cell culture flasks was replaced with 10 mL of the cell culture medium containing the test compound and the flasks were incubated for 1, 3, 6 or 24 h at 37°C / 5% CO_2. Afterwards the medium was removed and the cells were washed with phosphate-buffered saline pH 7.4. After trypsinization, the cell pellets were isolated by centrifugation, resuspended in 1–5 mL purified water, lysed by ultrasonication and appropriately diluted using purified water. The rhodium content of the samples was determined by AAS and the protein content was determined by the Bradford method as described in our recent publication in more detail [2]. The results were obtained in two independent experiments and were expressed as nmol rhodium per milligram of cellular protein.

3. Results

3.1. Chemistry

The NHC ligands of the target [(COD)(NHC)RhCl] complexes consist of a phenylimidazole core structure. In a first step of the synthesis procedure (see Scheme 1), the respective phenylimidazoles **1a** – **1d** were *N*-alkylated with ethyl iodide or 2-bromopropane, respectively, under basic conditions supported by 50 W microwave irradiation to yield the phenylimidazolium halides **2a** – **2h**. The [(COD)(NHC)RhCl] complexes of our previous projects had been prepared by reaction of the respective phenylimidazolium cations with half equivalent of silver iodide for 4h, followed by a transmetalation reaction of the formed silver intermediate using the rhodium dimer bis[chlorido(1,5-cyclooctadiene)rhodium(I)] for up to 18 h [2,3,35]. In this work, the same reaction mechanism was supported by microwave irradiation (50 W) leading overall to convenient reaction periods (up to 5 h in total) for the target complexes **3a** – **3h**. After removal of the formed silver halide by filtration, the pure products were obtained by chromatography over silica and precipitation as yellow solids in yields of 42 – 89%. The high purity of all target complexes was confirmed by elemental analyses and mass spectrometry confirmed the expected molecular ion $[M]^+$ signals for **3a** – **3h**.

In the ^{1}H-NMR spectra several diagnostic features can be observed, which clearly confirm coordination of the NHC ligand to the metal. Thus, the hydrogen signal at the C2 carbon of the imidazole structure (at approx. 9-11 ppm in the spectra of **2a** – **2h**) is absent and the hydrogen at C5 of the imidazole appears as singlet as a consequence of the missing long-range coupling to the absent C2 hydrogen. In the complexes with ethyl side chains, **3a** – **3d**, the CH_2 hydrogens appear as individual non-equivalent signals with dq splitting after metal coordination, which can be ascribed to rotations around the Rh-C bond [40]. In the ^{13}C-NMR spectra, the signals for the C2 carbon are significantly downfield-shifted in comparison with the respective signals of the imidazolium NHC precursors (180.5 – 183.5 ppm for **3a** – **3h** vs 133.4 – 137.4 ppm for **2a** – **2h**) and show a characteristic ^1J rhodium-carbon coupling of 50.6 – 51.5 Hz.

3.2. Cell growth inhibitory effects

For evaluation of cytotoxic effects by complexes **3a** – **3h** we selected three relevant adherent cancer cell lines, namely HT-29 colon adenocarcinoma, MDA-MB-231 breast carcinoma and MCF-7 breast adenocarcinoma. The cell growth inhibitory effects of **2a** – **2h** and **3a** – **3h** were determined and expressed as IC_{50} values (see Table 1). The metal free NHC ligand precursors **2a** – **2h** did not reach IC_{50} values up to a concentration of 100 µM and can therefore be considered as inactive, which is in strong agreement with our previous results obtained with some of the compounds

Table 1
Cell growth inhibition (IC_{50} values, µM) of **2a** – **2h** and **3a** – **3h** against selected cancer cell lines; the values between brackets represent the experimental errors.

	R1	R2	HT-29	MCF-7	MDA-MB-231
2a – 2h			>100	>100	>100
3a	-H	$-CH_2CH_3$	6.4 (±0.5)	4.5 (±0.4)	2.0 (±0.7)
3b	-F	$-CH_2CH_3$	6.7 (±1.9)	4.3 (±0.6)	2.4 (±0.3)
3c	-Cl	$-CH_2CH_3$	7.2 (±2.0)	4.9 (±2.0)	2.5 (±0.7)
3d	-Br	$-CH_2CH_3$	9.2 (±0.3)	5.1 (±0.5)	2.7 (±0.6)
3e	-H	$-CH(CH_3)_2$	3.1 (±0.6)	1.8 (±0.2)	1.2 (±0.2)
3f	-F	$-CH(CH_3)_2$	1.9 (±0.2)	1.7 (±0.1)	1.0 (±0.1)
3g	-Cl	$-CH(CH_3)_2$	4.1 (±0.9)	2.9 (±0.6)	1.4 (±0.2)
3h	-Br	$-CH(CH_3)_2$	2.9 (±1.0)	2.2 (±1.0)	1.4 (±0.6)

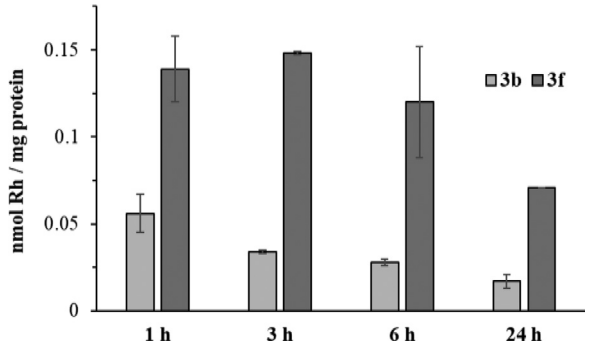


Fig. 2. Cellular rhodium levels (nmol Rh per milligram cellular protein) of HT-29 cells after exposure to 2.0 µM of **3b** or **3f**.

[39]. In contrast, all [(COD)(NHC)RhCl] complexes displayed good cell proliferation activity with IC_{50} values in the range of 0.9 to 9.2 µM. A clear preference for the isopropyl side chain over the ethyl side chain was evident, as in all cases the IC_{50} values of the isopropyl substituted complexes **3e** – **3h** were lower than that of the corresponding ethyl derivative (compare results for **3a** vs **3e, 3b** vs **3f, 3c** vs **3g**, and **3d** vs **3h**). Regarding the para-substituents on the phenyl ring of the NHC ligands, the differences in the IC_{50} values within **3a** – **3d** and **3e** – **3h**, respectively, were small. However, among the more active isopropyl derivatives the para fluorine compound **3f** turned out to be the most active complex against all studied cell lines and reached very promising IC_{50} values between 0.9 and 2.0 µM.

The most active derivative **3f** was subjected to further studies regarding toxic effects against cell layers with high confluency, in order to distinguish between proliferation inhibition and "direct" toxicity against cells. For this purpose, we used an assay setup that we had recently used to check for host cell toxicity before subjecting compounds to antiviral studies in infected host cells [35]. For this purpose, Caco-2 human colon adenocarcinoma and Calu-3 lung adenocarcinoma cells were grown until almost confluent layers and then exposed for 24 h to different concentrations of **3f**. Under these conditions **3f** triggered IC_{50} values of $2.50^{(\pm 0.12)}$ µM in Caco-2 cells and $3.34^{(\pm 0.93)}$ µM in Calu-3 cells. Although these values are higher than the IC_{50} values of **3f** for proliferation inhibition, the margin between toxicity against extended cell layers and proliferation inhibition is rather small and does not indicate a preference for proliferation inhibition over direct toxic effects against the cells.

3.3. Cellular uptake

In order to evaluate if the enhanced activity of the derivatives with the isopropyl side chain was a consequence of a higher lipophilicity resulting in a higher uptake of the complexes, the cellular rhodium levels of the most active complex, **3f**, were determined in comparison to those obtained with the ethyl analogue **3b** (see Fig. 2). For this purpose, HT-29 cells were exposed to 2.0 µM of **3b** or **3f** over periods of up to 24 h and the cellular rhodium levels were determined by high-resolution continuum source atomic absorption spectroscopy (HRCS-AAS).

Both complexes showed a rapid uptake within the first hour of incubation and decreased values after longer periods (24 h). In all cases the levels obtained with **3f** were significantly higher than those found with **3b**, which indicates that the higher cytotoxicity of the isopropyl derivatives could in fact be related to a higher accumulation in the cells.

4. Discussion and conclusions

A series of eight [(COD)(NHC)RhCl] complexes was prepared and studied as possible anticancer agents.

[(COD)(NHC)RhCl] complexes can be prepared by different synthesis procedures [41]. A convenient frequently used method is the procedure reported by Chianese et al. that goes back to the transmetalation method of Wang and Lin [42,43]. In the first step of this method, the imidazolium NHC precursor is treated with silver oxide leading to the formation of silver carbene complexes that can be reacted in a second step with the rhodium(I) dimer $[Rh(COD)Cl]_2$ to obtain the neutral target species of the type [(COD)(NHC)RhCl]. In our previous studies we had applied a one-pot version of this method without isolation of the silver NHC intermediates [2,3,35]. The time required for the transmetalation reaction depended on the nature of the NHC precursor. Extended reaction periods were necessary in particular in case of phenylimidazole-based NHC precursors [35]. Alternatively, [(COD)(NHC)RhCl] complexes can also be prepared by base catalysed one-pot methods [11,44].

In this work we have used a modified version of the previous one-pot procedure by applying 50 W microwave irradiation. Complexes **3a** and **3b** had already been reported and prepared according to the non-microwave procedure with a 4 h reaction with silver oxide followed by 18 h reaction with $[Rh(COD)Cl]_2$ at room temperature [35]. The microwave assisted procedure allowed to significantly shorten the reaction periods with 15 min for the treatment with silver oxide and 3 h for **3a** and 4 h for **3b**, respectively. In conclusion, the method might provide a suitable improvement for the accelerated synthetic access to [(COD)(NHC)RhCl] complexes from NHC precursors that otherwise require extended reaction periods. The microwave-assisted procedure does not yet represent an optimized method and in particular the effect of heating versus microwave irradiation would be worthwhile to evaluate.

The target compounds were studied for their antiproliferative effects in selected cancer cell lines and displayed strong cytotoxic effects with IC_{50} values in the micromolar range. There was a clear preference for the branched (isopropyl) side chains at the NHC nitrogen atoms versus the non-branched (ethyl) side chains. In previous studies, in which a benzimidazole based ligand was used instead of the phenylimidazole moiety, shorter methyl side chains were preferred over ethyl groups [2]. Within the more active isopropyl derivatives of this report, the compound with the fluorine substituent on the phenyl ring of the NHC ligand, **3f**, triggered the highest activity in all studied cell lines. Cellular uptake studies indicated that the enhanced activity of the isopropyl derivatives could primarily be a consequence of stronger accumulation in the cells. Taken together, this indicates that careful optimization of the NHC core structures in relation to the side chains at the NHC nitrogen atoms can be an useful strategy to fine-tune and optimize the antiproliferative effects of [(COD)(NHC)RhCl] complexes. With IC_{50} values between 0.9 and 2.0 µM, **3f** exceeded the activity of related monocarbene gold complexes of the type (NHC)AuCl [31]. Additional studies evaluating the toxicity of **3f** against non prolif-

erating cells indicated that the complex causes both proliferation inhibition and direct toxic effects against the cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2022.122300.

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