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Rhodium(I) N-heterocyclic carbene complexes: synthesis and cytotoxic properties†

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Rhodium(I) complexes bearing N-heterocyclic carbene (NHC) ligands have been widely used in catalytic chemistry, but there are very few reports of biological properties of these types of complexes. A series of benzimidazolium salts and their [RhCl(NHC)(COD)] complexes were synthesized. The obtained complexes were synthesized and characterized by elemental analysis, FT-IR, ^{1}H and ^{13}C NMR. All compounds were screened for *in vitro* cytotoxic activities against a panel of human cancer cells (HT-29 colon, Ishikawa endometrial, and U-87 glioblastoma) using the MTT assay for 48 h of incubation time. Mouse fibroblast cells (L-929) were used as healthy cells. Complexes had exhibited significantly higher cytotoxic activity towards cancer cells than their ligands and complex **2b** showed the most selective cytotoxic activity against HT-29 cancer cells (SI;7.05) and Ishikawa cancer cells (SI; more than 9.8). The complexes showed strong *in vitro* cytotoxic activity against cancer cells, with IC_{50} values of lower than 10 μM (except **2a** against HT-29 (12.8 μM) and **2b** against U-87 (11.1 μM)). All complexes (**2a**–**d**) showed the highest *in vitro* cytotoxic activity against Ishikawa endometrial cancer cells with IC_{50} values of 2.93 ± 0.06, <1, 2.60 ± 0.05, and 2.85 ± 0.06 μM, respectively. Complexes were found to be highly cytotoxic against HT-29, Ishikawa, and U-87 cancer cells compared to the anticancer agents, cisplatin and 5-FU.

1. Introduction

Ligand design is central to the development of new organometallic and coordination compounds as they control the overall properties, the activities and the reactivities of a metal center. Over recent years, N-heterocyclic carbenes (NHCs)[1,2] are an interesting class of ligands with donor properties similar to phosphines.[3] Since the discovery of the first N-heterocyclic carbene transition metal complexes by Wanzlic[4] and Öfele[5] in the 1960s and later the successful isolation of a free NHC by Arduengo in 1991,[6] NHCs have become increasingly important and the development of NHC metal complexes is now a well-established area of research.[7,8] The electronic and steric parameters of NHC complexes can be modified easily and they have greater stability towards air, moisture and heating when compared with phosphine analogs.

Organometallics bearing NHCs as ligands have been increasingly a focus for inorganic medicinal chemists.[9–11] The advantages of N-heterocyclic carbene ligands and their chemical versatility not only imply a wide variety of structural diversity and coordination modes, but also a capability to form stable complexes with a large number of transition metals with different oxidation states.[12,13] So far, metal-NHC complexes have been mostly studied as new antibacterial and anticancer agents. Although research on anti-infectives has been largely focused on silver(I)–NHC complexes,[14–21] gold(I)–NHC derivatives are the most studied examples concerning new anticancer agents.[18–26] Besides silver and gold, other metals including palladium,[27–29] platinum,[30–34] ruthenium[35–37] and rhodium[31] have also been used as bioactive complexes. However, the anti-cancer properties of rhodium(I)-N-heterocyclic carbene complexes have rarely been studied.[38–40] Rhodium(I) is isoelectronic with platinum(I), and rhodium(I)-NHC complexes have a square planar geometry like *cis*-platinum, a clinically used anticancer drug.

In a recent study, we have reported the synthesis of azolium salts and their silver(I), gold, ruthenium, and palladium complexes, which were tested as potential biologically active agents.[41–50] In this work, we report the synthesis and characterization of

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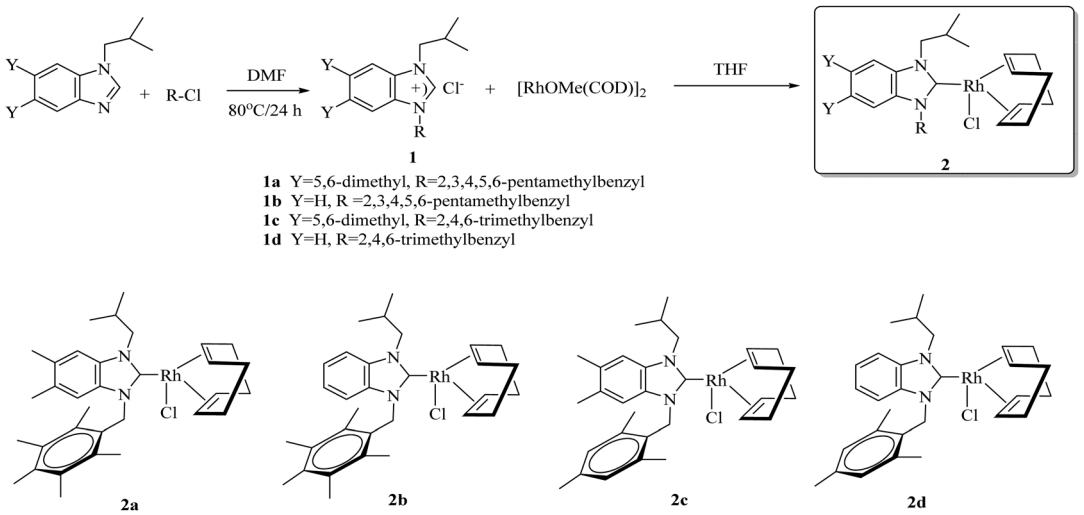

Scheme 1 Synthesis of rhodium–NHC complexes.

1,3-dialkylbenzimidazolium salts (**1a–d**) and novel benzimidazol-2-ylidene rhodium(I) (**2a–d**) complexes of the general formula [RhCl(NHC)(COD)] (**2a–d**) (Scheme 1). The cytotoxic properties of the synthesized benzimidazolium salts (**1a–d**) and rhodium complexes (**2a–d**) were tested against the human cancer cell lines, HT-29 colon, Ishikawa endometrial, and U-87 glioblastoma.

2. Materials and methods

2.1. Materials and measurements

All reactions for the preparation of benzimidazolium salts and their complexes were carried out under argon in flame-dried glassware using standard Schlenk techniques. Chemicals and solvents were purchased from Sigma-Aldrich, and Merck. The solvents used were purified by distillation over the drying agents indicated and were transferred under Ar:Et_2O, and THF (Na/K alloy), CH_2Cl_2 (P_4O_{10}), hexane, and toluene (Na). Melting points were measured in open capillary tubes with an Electrothermal-9200 melting point apparatus and are uncorrected. IR spectra were recorded using an ATR unit in the range of 400–4000 cm^{-1} with a PerkinElmer Spectrum 100 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded using a Varian As 400 Merkur spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane as an internal reference. Coupling constants (J values) are given in hertz. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, and m = multiplet signal.

2.2. General procedure for the synthesis of benzimidazolium salts

The following benzimidazolium salt (**1a–d**) precursors were synthesized according to the literature.[46,48]

The benzimidazolium salts **1a–d** were prepared by reacting 1-isobutylbenzimidazole (1 mmol) with various alkyl chlorides (1.1 mmol) in dimethylformamide (5 mL) at 80 °C and the resulting mixture was stirred for 24 hours (Scheme 1). Diethyl ether (15 mL) was added to obtain a white crystalline solid, which was subsequently filtered off. The solid was washed with diethyl ether (3 × 10 mL), dried under vacuum and the crude product was recrystallized from dichloromethane/diethyl ether (1 : 3 ratio).

2.2.1. 1-(isoButyl)-3-(2,3,4,5,6-pentamethylbenzyl)-5,6-dimethylbenzimidazolium chloride, (1a). Yield 93%, mp: 219.9 °C, ν(CN) = 1550 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.98 (d, 6H, $CH_{3(a,b)}$, J = 8 Hz), 2.14 (hept., 1H, $H_{2'}$, J = 8 Hz), 2.25 (s, 6H, $CH_{3(c,d)}$), 2.28 (s, 3H, $CH_{3(e)}$), 2.30 (s, 6H, $CH_{3(f,h)}$), 2.32 (s, 3H, $CH_{3(i)}$), 2.41 (s, 3H, $CH_{3(g)}$), 4.46 (d, 2H, $H_{1'}$, J = 8 Hz), 5.80 (s, 2H, $H_{1''}$), 7.05 (s, 1H, H_7), 7.38 (s, 1H, H_4), 10.48 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 16.9 ($C_{f,h}$), 17.1 ($C_{e,i}$), 17.3 (C_g), 19.7 ($C_{a,b}$), 20.6 ($C_{c,d}$), 28.7 ($C_{2'}$), 47.9 ($C_{1''}$), 54.1 ($C_{1'}$), 112.7 (C_4), 113.4 (C_7), 125.2 ($C_{5''}$), 129.9 (C_8), 130.43 (C_9), 133.8 ($C_{3'';7''}$), 133.5 ($C_{4'';6''}$), 136.8 (C_5), 136.9 (C_6), 137.1 ($C_{2''}$), 142.3 (C_2).

2.2.2. 1-(isoButyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride, (1b). Yield 92%, mp: 198.2 °C, ν(CN) = 1546 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) = 1.05 (d, 6H, $C_{H3\ (a,b)}$, J = 4 Hz), 2.24 (s, 6H, $CH_{3(c,g)}$), 2.28 (s, 6H, $CH_{3(d,f)}$), 2.28 (s, 3H, $CH_{3(e)}$), 2.38 (Hep, 1H, $H_{2'}$, J = 8 Hz), 4.51 (d, 2H, $H_{1'}$, J = 8 Hz), 5.94 (s, 2H, $H_{1''}$), 7.22–7.70 (m, 4H, $H_{4,5,6,7}$), 11.29 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) =16.9 ($C_{c,g}$), 17.1 ($C_{d,f}$), 17.3 (C_e), 19.7 ($C_{a,b}$), 28.8 ($C_{2'}$), 48.5 ($C_{1''}$), 54.2 ($C_{1'}$), 113.0 (C_4), 113. 8 (C_7), 125.1 ($C_{5''}$), 126.8 (C_5), 126.9 (C_6), 131.3 ($C_{4''}$), 131.8 ($C_{6''}$), 133.5 ($C_{3'';7''}$), 133.8 ($C_{8;9}$), 137.2 ($C_{2''}$), 143.7 (C_2).

2.2.3. 1-(isoButyl)-3-(2,4,6-trimethylbenzyl)-5,6-dimethylbenzimidazolium chloride (1c). Yield 89%, mp: 249.7 °C, ν(CN) = 1550 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) = 1.01 (d, 6H, $CH_{3(a,b)}$, J = 8 Hz), 2.29 (s, 3H, $CH_{3(c)}$), 2.30 (s, 3H, $CH_{3(d)}$), 2.32 (s, 6H, $CH_{3(e,g)}$), 2.40 (s, 3H, $CH_{3(f)}$), 2.44 (Hep, 1H, $H_{2'}$, J = 8 Hz), 4.40 (d, 2H, $H_{1'}$, J = 8 Hz), 5.84 (s, 2H, $H_{1''}$), 6.9–7.38 (m, 4H, $H_{4,7,4'',\ 6''}$), 11.34 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) = 19.7 ($C_{a,b}$), 20.2 ($C_{c,d}$), 20.6 (C_e), 20.8 (C_g), 21.0 (C_f), 28.7 ($C_{2'}$), 47.1 ($C_{1''}$), 54.1 ($C_{1'}$), 112.6 (C_4), 113.5 (C_7), 125.4 ($C_{4'';6''}$), 130.0 ($C_{3'';5'';7''}$), 137.0 ($C_{8;9}$), 137.8 ($C_{5;6}$), 139.5 ($C_{2''}$), 142.9 (C_2).

2.2.4. 1-(isoButyl)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride (1d). Yield 94%, $C_{21}H_{27}ClN_2$, M = 342.91 g mol^{-1}, mp: 218.3 °C, ν(CN) = 1469 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.00 (d, 6H, $CH_{3\ (a,b)}$, J = 8 Hz), 2.25 (s, 3H, $CH_{3(d)}$), 2.28 (s, 6H, $CH_{3(c,e)}$), 2.37 (hept, 1H, $H_{2'}$, J = 8 Hz), 4.42 (d, 2H, $H_{1'}$, J = 8 Hz), 5.90 (s, 2H, $H_{1''}$), 6.88 (s, 2H, $H_{4'',6''}$), 7.10 (d, 1H, H_4, J = 8 Hz), 7.37 (t, 1H, H_5, J = 8 Hz), 7.51(t, 1H, H_6, J = 8 Hz), 7.64(d, 1H, H_7, J = 8 Hz), 11.61 (s, 1H, H_2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 19.8 ($C_{(c,e)}$), 20.3 ($C_{(a,b)}$), 21.1($C_{(d)}$), 28.9 ($C_{2'}$), 47.5 ($C_{1''}$), 54.3 ($C_{1'}$), 113.1 (C_4), 114.0 (C_7), 125.2 ($C_{5;6}$), 126.9 ($C_{4''}$), 127.1 ($C_{6''}$), 130.2 (C_8), 131.3 (C_9), 131.8 ($C_{3''}$), 137.9 ($C_{5'';7''}$), 139.6 ($C_{2''}$), 144.2 (C_2).

2.3. General procedure for the synthesis of the rhodium carbene complexes

A solution of benzimidazolium salts (**1a–d**) (10 mmol) and rhodium dimer [RhOMe(COD)]$_2$ (5 mmol) in THF (15 mL) was stirred and heated under reflux for 5 h. Upon cooling to room temperature, yellow crystals of (**2a–d**) were obtained. The crystals were filtered, washed with diethyl ether (2 × 15 mL) and dried under vacuum. The crude product was recrystallized from CH_2Cl_2/Et_2O.

2.3.1. Chloro[1-isobutyl-3-(2.3.4.5.6-pentamethylbenzyl)-5,6-dimethylbenzimidazole-2-ylidene]rhodium(I) 2a. Yield 78%, mp: 228.6 °C, ν(CN) = 1450 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.07 (d, 6H, $CH_{3\ (a,b)}$ J = 8 Hz), 1.94 (d, 4H, CH_2COD, $H_{2''',3'''}$, J = 8 Hz), 2.23 (s, 3H, $CH_{3(g)}$), 2.25 (s, 6H, $CH_{3(c,d)}$), 2.30 (m, 12H, $CH_{3(e,f,h,i)}$), 2.35–2.55 (m, 4H, CH_2COD, $H_{6''',7'''}$), 2.96 (Hep, 1H, $H_{2'}$, J = 8 Hz), 3.47 (d, 2H, CHCOD, $H_{4''',5'''}$, J = 8 Hz), 4.51 (d, 2H, CHCOD, $H_{1''',8'''}$, J = 8 Hz), 5.12 (d, 2H, $H_{1'}$, J = 8 Hz), 5.91 (m, 2H, $H_{1''}$), 6.40–7.26 (m, 2H, $H_{4,7}$). ^{13}CNMR ($CDCl_3$, 100 MHz) δ (ppm) 16.8 ($C_{f,h}$), 17.2 ($C_{e,i}$), 17.3 (C_g), 20.1 (C_a), 20.3 (C_b), 20.6 (C_c), 20.9 (C_d), 28.3 ($C_{2'}$), 29.2 ($C_{6''}$), 29.3 ($C_{7'''}$), 32.5 ($C_{3'''}$), 33.3 ($C_{2'''}$), 51.2 ($C_{1''}$), 55.6 ($C_{1'}$), 99.0 ($C_{4'''}$), 99.1 ($C_{5'''}$), 99.1 ($C_{1'''}$), 99.2 ($C_{8'''}$), 110.2 (C_4), 111.8 (C_7), 125.2 ($C_{5''}$), 128.6 ($C_{8;9}$), 130.3 ($C_{3'';7''}$), 130.4 ($C_{4'';6''}$), 132.8 ($C_{5;6}$), 135.6 ($C_{2'',5''}$), 194.8 (d, J =50.1Hz) (C_2).

2.3.2. Chloro[1-isobutyl-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazole-2-ylidene]rhodium(I) 2b. Yield 80%, mp: 225.6 °C, ν(CN) = 1483 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.08 (d, 6H, $CH_{3\ (a,b)}$ J = 8 Hz), 1.86–2.13 (m, 4H, CH_2COD, $H_{2''',3'''}$), 2.26 (s, 12H, $CH_{3(c,d,f,g)}$), 2.32 (s, 3H, $CH_{3(e)}$), 2.36–2.60 (m, 4H, CH_2COD, $H_{6''',7'''}$), 2.99 (Hept, 1H, $H_{2'}$ J = 8 Hz), 3.49 (d, 2H, CHCOD, $H_{4''',5''}$ J = 8 Hz′), 4.54 (d, 2H, CHCOD, $H_{1''',8'''}$ J = 8 Hz), 5.15 (d, 2H, $H_{1'}$, J = 8 Hz), 6.04 (s, 2H, $H_{1''}$), 6.46–7.26 (m, 4H, $H_{4,5,6,7}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 16.8 ($C_{c,g}$), 17.2 ($C_{d,f}$), 17.3 (C_e), 20.7 (C_a), 21.0 (C_a), 28.3 ($C_{2'}$), 29.2 ($C_{6'''}$), 29.5 ($C_{7'''}$), 32.4 ($C_{3'''}$), 33.3 ($C_{2'''}$), 51.6 ($C_{1''}$), 55.7 ($C_{1'}$), 99.4 ($C_{4''',5'''}$), 99.6 ($C_{1''',8'''}$), 109.8 (C_4), 111.1 (C_7), 121.4 (C_5), 122.0 (C_6), 128.4 ($C_{4'',6''}$), 133.0 ($C_{3'';7''}$), 135.0 (C_8), 135.6 (C_9), 135.7 ($C_{2'',5''}$), 196.7 (d, J =50.2 Hz) (C_2).

2.3.3. Chloro[1-(isobutyl)-3-(2,4,6-trimethylbenzyl)-5,6-dimethylbenzimidazole-2-ylidene]rhodium(I) 2c. Yield 87%, mp: 209.3 °C, ν(CN) = 1433 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.07 (d, 6H, $CH_{3\ (a,b)}$, J = 8 Hz), 1.79–2.13 (m, 4H, CH_2COD, $H_{2''',3'''}$), 2.21 (d, 6H, $CH_{3(c,d)}$, J = 8 Hz), 2.33–2.56 (m, 4H, CH_2COD, $H_{6''',7'''}$), 2.89 (Hep, 1H, $H_{2'}$, J = 8 Hz), 3.31 (d, 2H, CHCOD, $H_{4''',5'''}$, J = 8 Hz), 3.80 (s, 9H, $CH_{3(e,f,g)}$), 4.73 (d, 2H, CHCOD, $H_{1''',8'''}$, J = 8 Hz), 5.14 (d, 2H, $H_{1'}$, J = 8 Hz), 6.06 (s, 2H, $H_{1''}$), 6.74 (s, 2H, $H_{3'',7''}$), 7.26 (s, 2H, $H_{4,7}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 20.2 (C_c), 20.2 (C_d), 20.6 (C_a), 20.8 (C_b), 28.1 ($C_{2'}$), 29.1, ($C_{6''}$), 29.5 ($C_{7'''}$), 32.2($C_{3'''}$), 33.5 ($C_{2'''}$), 53.1 ($C_{1''}$), 55.6 ($C_{1'}$), 56.5 ($C_{e;g}$), 60.8 (C_f), 99.5 ($C_{4'''}$), 99.5 ($C_{5'''}$), 99.9 ($C_{1'''}$), 100.0 ($C_{8'''}$), 104.8 ($C_{3'';7''}$), 110.7 (C_4), 111.3 (C_7), 128.8, 131.1 ($C_{2''}$), 131.9 ($C_{8,9}$), 132.9 (C_5), 134.4 (C_5), 137.4 ($C_{5''}$), 153.4 ($C_{4'',6''}$), 194.9 (d, J = 50.0 Hz) (C_2).

2.3.4. Chloro[1-((isobutyl)-3-(2,4,6-trimethylbenzyl) benzimidazole-2-ylidene] rhodium(I) 2d. Yield 79%, mp: 206.1 °C, ν(CN) = 1452 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.05 (d, 6H, $CH_{3(a,b)}$, J = 8 Hz, J = 8 Hz), 1.85–2.15 (m, 4H, CH_2COD, $H_{2''',3'''}$), 2.26–2.59 (m, 4H, CH_2COD, $H_{6''',7'''}$), 2.99 (Hep, 1H, $H_{2'}$), 3.32 (d, 2H, CHCOD, $H_{4''',5''}$, J = 8 Hz′), 3.80 (d, 9H, $CH_{3(c,d,e)}$), 4.58 (d, 2H, CHCOD, $H_{1''',8'''}$, J = 8 Hz), 5.16 (d, 2H, $H_{1'}$, J = 8 Hz), 6.07 (s, 2H, $H_{1''}$), 6.75 (s, 2H, $H_{3'',7''}$), 7.03–7.34 (m, 4H, $H_{4,5,6,7}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 20.6 (C_a), 20.8 (C_b), 28.1 ($C_{2'}$), 29.2, ($C_{6''}$), 29.5 ($C_{7'''}$), 32.1($C_{3'''}$), 33.6 ($C_{2''}$), 53.4 ($C_{1''}$), 55.7 (C_1), 56.4 ($C_{c;e}$), 60.8 (C_d), 99.8 ($C_{4'''}$), 99.9 ($C_{5'''}$), 100.3 ($C_{1'''}$), 100.3 ($C_{8'''}$), 104.8 ($C_{3'';7''}$), 110.2 (C_4), 111.0 (C_7), 127.2 ($C_{5,6}$), 131.6 (C_8), 134.3 (C_9), 135.8, ($C_{2''}$), 137.4 ($C_{5''}$), 153.2 ($C_{4'',6''}$), 196.8 (d, J = 50.2 Hz) (C_2).

2.3. Cell lines and culture conditions

Human colorectal adenocarcinoma HT-29 (ATCC® HTB-38™), human brain glioblastoma U-87 (ATCC® HTB-14™), FBS (ATTC, FBS, 30-2020), RPMI-1640 media (ATTC, 30-2001), and 100 units per mL penicillin and 100 mg mL^{-1} streptomycin (ATTC, 30-2300) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Human endometrial adenocarcinoma Ishikawa (ECACC, 99040201) and L-929 mouse adipose fibroblast cells L929(NCTC) (ECACC 85103115) were supplied by European Collection of Authenticated Cell Cultures (ECACC) (Salisbury, UK). Dulbecco's modified Eagle's medium (DMEM, D6429), MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; M-2128) and trypsin–EDTA solution (T-3924) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Deutschland). The cells were grown in T-75 flasks in an incubator with 5% CO_2, 95% humidity, at 37 °C using a culture medium, supplemented with 10% fetal bovine serum and antibiotics. HT-29, Ishikawa, and U-87 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), and L-929 healthy cells were cultured in RPMI-1640 medium. All cell lines were subcultured when they reached 70–80% confluency.

2.3.1 Cell viability assay. The *in vitro* cytotoxic activities of the salts (**1a–d**) and Rh complexes (**2a–d**) toward cancer cell lines (HT-29, Ishikawa, and U-87) were evaluated with the MTT assay.[51] In brief, 1 × 10^5 cells per mL were seeded in 96-well microplates in respective media (100 μL) and incubated at 37 °C in a 5% CO_2 atmosphere under the 95% humidified condition. After incubation for 24 h for the cell attachment, the cells were treated with different concentrations of the salts (1–100 μM) and complexes (1–30 μM) and incubated for 48 h. All compounds were dissolved in DMSO and diluted in the complete culture medium (DMSO concentration in each well would not exceed 0.5%).

The same amount of DMSO was added to the control wells. At the end of the incubation time, 10 μL MTT (5 mg mL^{-1}, in PBS, pH 7.2) was added to each well. After incubation for 2 h with an MTT solution at 37 °C, the medium was removed and 100 μL of DMSO was added. The cell viability was analyzed by measuring the absorbance at 570 nm using a BioTek plate reader (BioTek, Epoch, USA).

2.4. Statistical analysis

The data presented here are means of at least three independent experiments; in a single experiment, each concentration was assayed in triplicate. All results are expressed as means ± SD. Data were analyzed using one-way analysis of variance and differences were considered significant at $^{*}p < 0.05$, $^{**}p < 0.005$, $^{\#}p < 0.0005$, and $^{\#\#}p < 0.0001$. IC_{50} values (drug concentrations responsible for 50% cell growth inhibition) were calculated by GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1. Synthesis of benzimidazolium salts and their rhodium(I) complexes

According to the literature,[46,48] ligand precursors (**1a–1d**) were synthesized by quaternization of 1-isobutyl-benzimidazole in DMF with the corresponding benzyl chlorides in nearly quantitative yield, 89–94% (Scheme 1). The salts are stable in solid-state and solution in air and moisture. All salts are soluble in chlorinated solvents, alcohols, and water. The NCHN protons appear in the ^{1}H NMR range of **1a–1d** at 10.48, 11.29, 11.34 and 11.61 ppm, respectively, and these downfield signals confirm the formation of the benzimidazolium salts. The ^{1}H-NMR shifts of **1b–1d** are identical to those of the other characterized benzimidazolium salts.[18,20,21]

NHC-Rh complexes are prepared using a variety of methods: (i) reaction of a free carbene with dimeric precursor $[Rh(COD)Cl_2]_2$,[52] (ii) *in situ* deprotonation of azolium salts with a rhodium dimer $[Rh(OMe)COD]_2$,[53] (iii) reaction of electron-rich enetetramines with $[Rh(COD)Cl_2]_2$ under C=C bond cleavage,[54] (iv) transfer of the carbene unit from the silver(I)–NHC complex to the rhodium metal.[55] We chose the second preparation pathway for the synthesis of Rh(I)–NHC complexes. The advantage of this route is that no pre-generation of the free carbene is necessary and the methoxy ligands are protonated to give methanol upon reaction with the benzimidazolium salt. Rhodium N-heterocyclic carbene complexes **2a–d** were prepared by treatment of rhodium dimer $[Rh(OMe)COD]_2$ with two equivalents of 1,3-dialkylbenzimidazolium chloride salts in tetrahydrofuran under reflux. The complexes **2a–d** were obtained as yellow-orange crystalline solids in 78–87% yields (Scheme 1).

The new Rh complexes are very stable in the solid-state and have been characterized by ^{1}H and ^{13}C-NMR spectroscopy and FTIR. They show a characteristic (NCN) band at 1450, 1483, 1433 and 1452 cm^{-1} for **2a–d**, respectively. The formation of Rh(I)–NHC complexes **2a–d** was confirmed by the loss of the resonance signals of benzimidazolium C2 proton at 10.48, 11.29, 11.34 and 11.61 ppm and C2 carbon at 142.3, 143.7, 142.9 and 144.2 ppm for **1a–d,** respectively, in ^{1}H NMR and ^{13}C NMR spectra. The ^{13}C-NMR chemical shifts, which provide a useful diagnostic tool for metal carbene complexes, show that $C_{carbene}$ is substantially deshielded. The resonance of the carbene carbon of rhodium complexes was observed as a doublet at 194.8, 196.7, 194.9 and 196.8 ppm; and coupling constants $J(^{103}Rh–^{13}C)$ were 50.1, 50.2, 50.0 and 50.2 Hz for **2a–d,** respectively, in ^{13}C NMR spectra (see ESI†). These values were similar to those found for other rhodium(I) carbene complexes.[56–58]

3.2. Cytotoxic activity

Salts (**1a–d**) and Rh complexes (**2a–d**) were screened for their *in vitro* anticancer activities on HT-29, Ishikawa, and U-87 cancer cell lines using the MTT test in the different concentration range. After 48 h of treatment, a significant reduction in cell survival was observed depending on the concentration of the compounds and cell line type as shown in Fig. 1(a–h). The IC_{50} values (the half-maximal inhibitory concentration) of the salts (**1a–d**) and Rh complexes (**2a–d**) are given in Table 1.

The IC_{50s} for complexes (**2a–d**) were higher in healthy cells compared to the HT-29 human colon adenocarcinoma cells and Ishikawa human endometrial adenocarcinoma, suggesting that complexes possessed noteworthy selectivity for these cells. The selectivity index was calculated by dividing the IC_{50} values of the compound into healthy cells by the IC_{50} value of the same compound in cancer cells. Compounds with SI values of 3 and above represent more toxicity towards cancer cells compared to healthy cells. SI values of complexes (**2a–d**) against HT-29 and Ishikawa cells were found to be <1, 7.05, 2.31, 3.11 and 3, >9.8, 7.04, 4.26, and 2.20, respectively. Ishikawa cells were the most sensitive to compounds displaying the IC_{50} range from <1 to 2.60 ± 0.05 μM. The most important result was obtained with the complex **2b** exhibiting the highest *in vitro* cytotoxic activity against the Ishikawa and HT-29 cancer cells, with IC_{50} values <1 μM and 1.39 ± 0.05 μM, respectively. The results showed that complex **2b** was the most selectively toxic towards Ishikawa and HT-29 cancer cells with SI values >9.8 and 7.05, respectively. Complexes **2a** and **2c** were also determined to be toxic towards Ishikawa endometrium cancer cells with SI values of 3 (IC_{50}: 2.93 ± 0.06 μM) and 4.26 (IC_{50}: 2.60 ± 0.05 μM), respectively. Complex **2d** was determined to be toxic against HT-29 colon cancer cells with SI values of 3.11 (IC_{50}: 2.02 ± 0.03 μM). As the SI values of complexes (**2a–d**) against U-87 glioblastoma cells were lower than 2, they did not show significant selective *in vitro* cytotoxic activity on U-87 cells. Our result also indicated that complexes (**2a–d**) have greater selectivity (SI values of from <1 to >9.8) against cancer cells than cisplatin and 5-FU (SI values of from <1 to 1.27). Complexes did not have greater cytotoxic activity than 5-FU against U-87 glioblastoma cells and cisplatin against L-929 cells. Complexes display greater cytotoxic activity than cisplatin against HT-29 colon cancer cells (with 1.24, 11.24-fold, 3.32-fold, 7.87-fold, **2a**, **2b**, **2c**, and **2d**, respectively), Ishikawa endometrial cancer cells

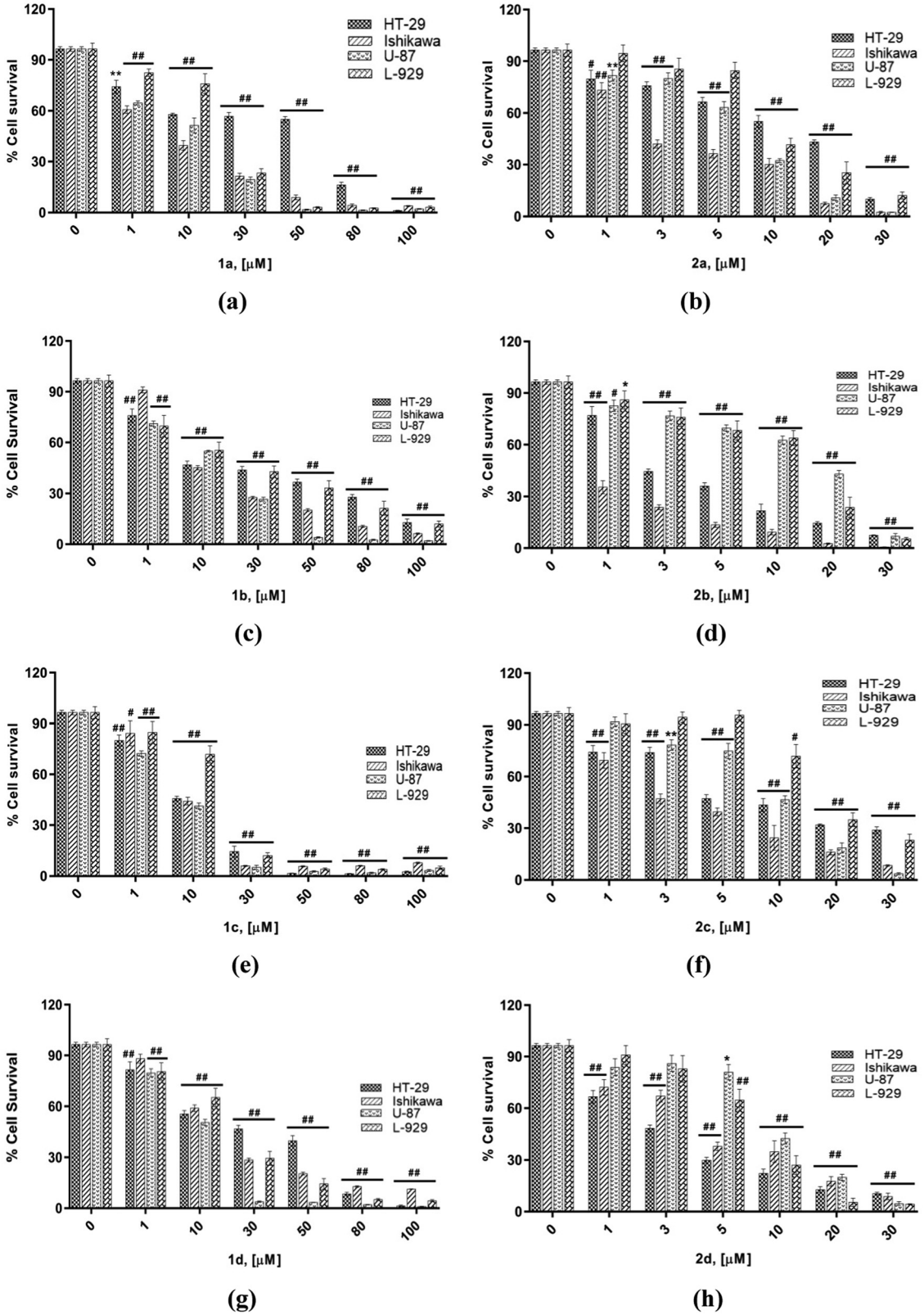

Fig. 1 Dose-dependent *in vitro* cytotoxic activities of the ligands (**1a**–**d**) and Rh complexes (**2a**–**d**) on HT-29 colon, Ishikawa endometrial, U-87 glioblastoma and L-929 healthy cells of 48 h incubation (n = 9). Data are representative of the mean of three separate experiments and are reported at the ±SD. (*$p < 0.05$, **$p < 0.005$, $^{\#}p < 0.0005$, $^{\#\#}p < 0.0001$ *vs.* control).

Table 1 *In vitro* cytotoxic activities of compounds for 48 h of incubation time

Compounds	IC_{50} (μM)[a] HT-29	Ishikawa	U-87	L-929[b]	SI^{HT-29}	SI^{ISH}	SI^{U-87}
Salts							
1a	71.9 ± 0.03	7.80 ± 0.08	19.6 ± 0.04	26.3 ± 0.98	<1	3.37	1.34
1b	9.58 ± 0.05	12.5 ± 0.03	21.9 ± 0.02	17.9 ± 0.04	1.86	1.43	<1
1c	12.3 ± 0.02	4.71 ± 0.07	9.73 ± 0.06	13.6 ± 0.01	1.11	2.88	1.39
1d	14.6 ± 0.06	9.36 ± 0.12	10.2 ± 0.02	14.7 ± 0.03	1.00	1.57	1.44
Complexes							
2a	12.8 ± 0.02	2.93 ± 0.06	6.59 ± 0.02	8.78 ± 0.04	<1	3	1.33
2b	1.39 ± 0.05	<1	11.5 ± 0.04	9.81 ± 0.02	7.05	>9.8	<1
2c	4.79 ± 0.06	2.60 ± 0.05	8.24 ± 0.02	11.1 ± 0.09	2.31	4.26	1.34
2d	2.02 ± 0.03	2.85 ± 0.06	6.81 ± 0.02	6.30 ± 0.01	3.11	2.20	<1
Cisplatin[c]	15.9 ± 1.27[59]	13[60]	36.2 ± 1.55[61]	16.5 ± 2.38[62]	1.03	1.27	<1
5-FU[d]	>100	>100	8.22 ± 0.37[63]	0.55 ± 0.04[64]	<1	<1	<1

[a] IC_{50} ± SD values are determined by the MTT assay. SD, standard deviation. [b] Healthy cells (mouse adipose fibroblast). [c] Cisplatin and [d]5-FU, 5-fluorouracil were standard drugs. See the Experimental section for full details. HT-29,; human colon adenocarcinoma; Ishikawa, human endometrial adenocarcinoma; U-87, human brain glioblastoma. [d] SI, selectivity index calculated [IC_{50} for healthy cell]/[IC_{50} for cancer cell].

(with 4.43-fold, >13-fold, 5-fold, 4.56-fold, **2a**, **2b**, **2c**, and **2d**, respectively), and U-87 glioblastoma cells (5.49-fold, 3.14-fold, 4.40-fold, 5.31-fold, **2a**, **2b**, **2c**, and **2d**, respectively). Complexes have been found to have quite high cytotoxic activity on HT-29 colon cancer cells (with >7.8-fold, >72-fold, >3.32-fold, <49.5-fold, **2a**, **2b**, **2c**, and **2d**, respectively) and Ishikawa cancer cells (with >34.3-fold, >100-fold, >38.5-fold, >35.1-fold, **2a**, **2b**, **2c**, and **2d**, respectively) compared to 5-FU.

Although Pt-based cytotoxic agents, such as cisplatin, carboplatin, and oxaliplatin, are effective in treating different types of cancer, their use has been limited because of their high toxicities.[65] Recent relevant studies are based on testing transition metal complexes other than platinum(II). The anticancer properties of some Rh(II) and Rh(III) complexes have also been evaluated. Researchers designed, synthesized and investigated the cytotoxic activity of Rh(III) complexes with thiabendazole and *N*-benzyl-thiabendazole ligands. Cytotoxic activity of complexes was determined against A549 human lung and SW480 human colon cancer cells using the MTT assay for 24 h of incubation. IC_{50} values of Rh-complexes (IC_{50s} for A549 10.3 ± 0.1 and 4.1 ± 0.2 μM; IC_{50s} for SW480 7.7 ± 0.4 and 3.3 ± 0.1 μM) were found to be lower than cisplatin against both cell lines. It was also determined that bearing the *N*-benzyl-thiabendazole ligand exhibits higher cytotoxic activity than bearing thiabendazole ligands.[66]

Esteghamat-Panah *et al.* synthesized a mononuclear Rh(III) complex and characterized it by elemental analysis and spectroscopic techniques with the bzimpy ligand in the *in vitro* anticancer activity of the ligand and the complex was assessed against human breast (MCF-7), leukemia (K562), and colorectal (HT-29) cancer cells by the MTT assay. Results showed that the complex was more cytotoxic than the bzimpy ligand (IC_{50s} for the bzimpy ligand and the Rh-complex, 114.66, 130.40, 95.74 and 8.14, 19.40, 43.02 against MCF-7, K562, and HT-29, respectively) and has the highest cytotoxic effect on MCF-7 breast cancer cells, also the complex was more cytotoxic against MCF-7 and HT-29 cancer cells than cisplatin.[67] Yellol *et al.* synthesized benzimidazole Ru, Ir and Rh cyclometalated complexes and screened for their cytotoxic activity against HT29, T47D, A2780 and A2780cisR cancer cell lines. They had reported that the cytotoxic activity of complexes depends on the metal center. The IC_{50} values of the Rh-complex with the methyl ligand were found to be >50, 8.97, 8.05 and 5.27 μM and those of the Rh-complex with the benzyl ligand were found to be 5.37, 22, 6.64 and 4.36 μM for HT29, T47D, A2780 and A2780cisR cancer cells, respectively. Researchers also found that the cytotoxicity of the Rh-complexes against the HT-29, T47D and A2780cisR cell lines is higher than that of the widely used drug, cisplatin, under similar conditions.[68]

4. Conclusions

In the present study, a series of 1,3-dialkylbenzimidazolium salts and their [RhCl(NHC)(COD)] complexes were synthesized and characterized by an appropriate method. The *in vitro* cytotoxic effect of the new ligands and Rh-NHC complexes was evaluated against the human colon (HT-29), endometrial (Ishikawa) and glioblastoma (U-87) cancer cells. Rh-NHC complexes showed larger *in vitro* cytotoxic effects than ligands on HT-29, Ishikawa and U-87 cells. Complex **2b** showed a significant selectivity index (SI) for Ishikawa (>9.8) and HT-29 (7.05) cancer cells. Furthermore, the IC_{50} values suggest that the Rh-complex is a potential anticancer candidate against the HT-29 and especially Ishikawa cell lines. Results also showed that complexes were more cytotoxic against cancer cells than cisplatin and 5-FU which are widely used chemotherapeutic drugs.

Conflicts of interest

There are no conflicts to declare.

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