

Research Article

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Novel ruthenium(II) N-heterocyclic carbene complexes: Synthesis, characterization, and evaluation of their biological activities

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Abstract: A series of ruthenium(II) complexes with N-heterocyclic carbene (NHC) ligands of the general type (arene)(NHC)Ru(II)X₂ (where X = halide) (**3a–3d**) were synthesized and characterized in order to compare their antibacterial activities with benzimidazolium salts **2**. Our comparison revealed that ruthenium(II) NHC complexes **3** were more active than benzimidazolium salts **2**. Furthermore, the two complexes **3b** and **3d** had a potent inhibitory effect against acetylcholinesterase with an IC₅₀ of 4.52 and 4.04 g·mL^{−1} and against tyrosinase with an IC₅₀ of 20.77 and 25.84 g·mL^{−1}, respectively. In addition, screening of benzimidazolium salts (**2a–2d**) and their ruthenium(II) complexes (**3a–3d**) against colon carcinoma cell lines (HCT-116) and hepatocellular carcinoma cell lines (HepG-2) were studied. The obtained results revealed that complex **3a** is the most active against vinblastines.

Keywords: biological activity, benzimidazolium salts, ruthenium complexes, silver complexes, HCT-116, HepG-2

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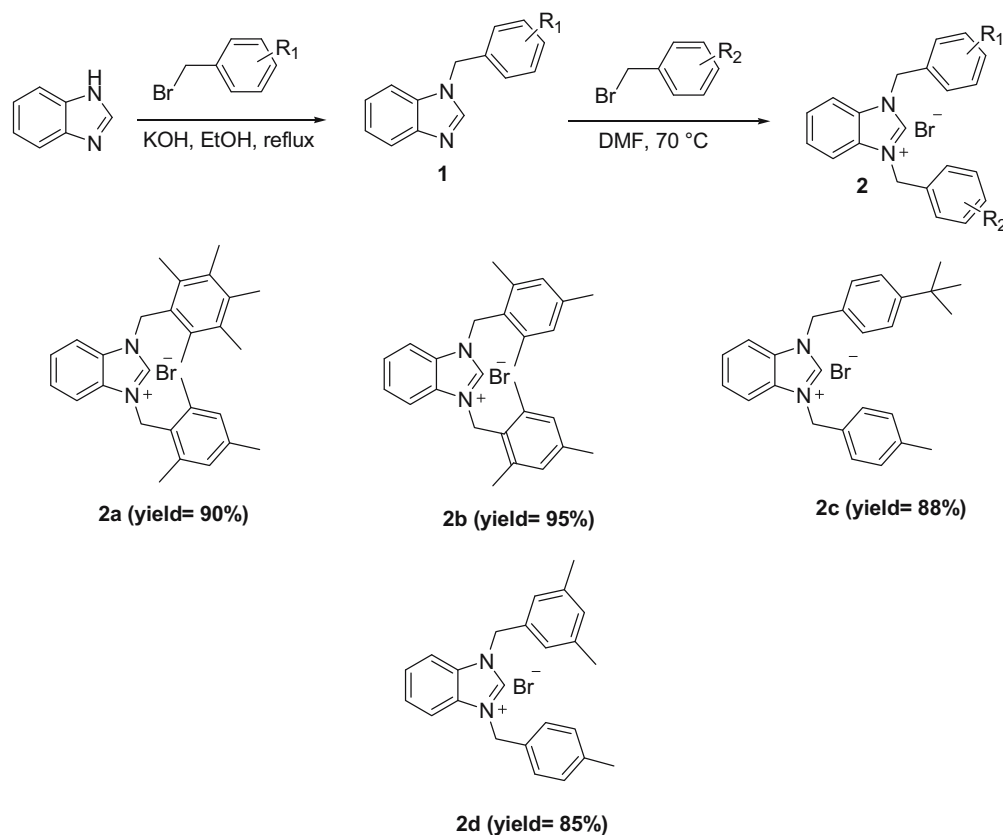
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1 Introduction

In modern medicine, the use of inorganic and organometallic substances is widespread (Rosenberg et al., 1969; Medici et al., 2015). N-Heterocyclic carbene (NHC) complexes are the new promising members of organometallic complexes for drug formulation (Noffke et al., 2012; Arduengo et al., 1991). The first study on the biological activities of NHC complexes was reported by Çetinkaya (Herrmann, 2002; Barnard et al., 2004) and co-workers. Several research groups, including ours, have synthesized functionalized NHC complexes and explored their biological properties for this purpose (Garner et al., 2015; Oehninger et al., 2013). Some studies showed that the main target of palladium NHCs in cancer cells is DNA similar to cisplatin (Ray, 2007). In this regard, ruthenium(II/III) complexes have been extensively investigated as anticancer and antimicrobial drugs as well as DNA binding agents (Vuradi et al., 2020). In particular, in the design of innovative anticancer drugs, ruthenium type complexes have special consideration and have been evaluated against different cancer cell lines as promising alternates for the well-known Pd(II) (cisplatin) and its derivatives (Ezhilarasu and Balasubramanian, 2018). Ruthenium possesses +2 and +3 oxidation states at physiological conditions and can bind to proteins, nucleic acids, sulfur, or oxygen-containing compounds in the cells (Jakupc et al., 2008; Keene et al., 2009). Furthermore, the interaction kinetics of ruthenium complexes with the cell components can be optimized depending on the nature of their ligand. This permits the ligand exchange rates of ruthenium complexes to be close to those of cellular processes, which makes them well-adapted for various medicinal applications. Therefore, compared with platinum-based drugs, ruthenium compounds might exhibit a higher cytostatic activity against various cancer cells and could be effective in the cells resistant to cisplatin. In addition, ruthenium complexes have emerged as a new and very interesting class of biologically active agents.

On several cancer cell lines, our research team is also examining metal complexes with anticancer properties (Slimani *et al.*, 2020; Karaca *et al.*, 2021). NHCs are easily synthesized, chemically modified, and exhibit superior properties ligands. The lipophilic end is essential in drug molecules, and to have this lipophilic end on NHC, it needs to be modified chemically. Thus, easy chemical modification of NHCs to have lipophilic ends in NHC-based drug molecules is significant. The NHCs can form a strong bond with the metal centers that lead to a more stable complex under moisture, heat, and air conditions. Due to these superior features, NHCs play an essential role in catalysis, biomedical applications, and functional material applications. Ruthenium-based complexes were used in medical applications due to low toxicity and are more capable of overcoming cancer cells' resistance than Pt-based drugs. The benefits of Ru complexes in biological applications were reported by different groups (Meier-Menches *et al.*, 2020). The most prominent feature of ruthenium in these studies is that it imitates iron elements in binding to biological molecules such as albumin and transferrin. Burgos *et al.* investigated antioxidant/prooxidant activities

and toxicity of some of the ruthenium–arene complexes. They concluded that Ru(II)–arene complexes behave as oxidants at low concentrations and as prooxidants at high concentrations. However, they reported that the ruthenium complexes could not negatively affect the Zebrafish embryos. Therefore, Ru(II)–arene complexes can be considered nontoxic. Among the NHCs, benzimidazole-based silver, gold, and ruthenium–NHC complexes have been studied intensely due to the benzimidazole structure being a component of many biological structures. In our previous study, Ru–NHC complexes showed good antiparasitical and antiproliferative activities (Al Nasr *et al.*, 2023). Encouraged by these results, we thought it would be helpful to examine the biological activities of some similar ruthenium complexes against different types of cancer cell lines and bacteria to determine the affinity between them. Herein, we synthesize and characterize a series of benzimidazole-containing Ru(II)–NHC complexes. Several spectroscopic and analytical techniques were used to characterize the novel compounds. Then, studies of their various biological properties, including antibacterial, cytotoxic, acetylcholinesterase (AChE) and inhibition, and antiproliferative, were conducted.



Scheme 1: General preparation and structures of benzimidazolium salts (2a–2d).

2 Results

2.1 Preparation of benzimidazolium salts 2a–d

The benzimidazolium salts (**2a–d**) as NHC precursors were synthesized as previously described (Ciftci et al., 2011). The structures of benzimidazolium salts **2a–d** were verified by ^1H NMR, ^{13}C NMR, FT-IR, and elemental analyses. Scheme 1 summarizes the synthesis of the 1,3-dialkylbenzimidazolium salts **2a–d**.

Due to the positive charge of the molecule, salts **2a–2d** show a low-field shift in the ^1H NMR spectra at 9.85–11.83 ppm characterizing the NCHN proton range. The ^1H NMR spectra showed resonance for the proton C(2)–H position of benzimidazolium salts **2a–2d** as sharp singlets at 10.85, 10.35, 11.62, and 11.75 ppm, respectively. In the ^{13}C NMR spectrum, the imino carbon occurs as typical singlet forms in the ^1H decoupled mode at 141.3, 143.0, 143.1, and 152.8 ppm, respectively, for benzimidazolium **2a–d**. Other signals include singlets at 20.76–35.18 ppm, attributable to the resonance of aliphatic carbon nuclei. The aromatic region shows a spectrum of peaks in the range of 113.38–152.82 ppm. These values are in perfect agreement with previously reported results (Antonarakis and Emadi, 2010; Bilel et al., 2014; Briguglio et al., 2015; Çetinkaya et al., 1996; Ciurea et al., 2020; Crump et al., 2004; Hartinger et al., 2013; He et al., 2016; Keppler and Rupp, 1986; Pizarro-Cerdá and Cossart, 2019).

2.2 Preparation of ruthenium–carbene complexes 3a–3d

Various methods have been described for the preparation of Ru(II)–NHC complexes (**3a–d**). In this regard, transmetalation with Ag–NHC complexes has proven to be the most useful used as a carbene transfer agent in order to avoid complicated working difficulties (Moore and Flaws, 2011).

The synthesis of (*p*-cymene) NHC Ru(II)Cl₂ was achievable through a well-known two-step procedure (Scheme 2). Benzimidazoles **1a–1d** were alkylated with benzyl bromide in the first step, resulting in the synthesis of the benzimidazolium bromides **2a–2d**. Then, the activation of salts **2a–2d** with silver oxide and their subsequent transmetalation with [(*p*-Cym)RuCl₂] gave the target complexes [RuCl₂(*p*-cymene) (NHC)] (**3a–3d**) with a good yield (85–95%).

The methylic protons of complexes **3a–3d** appeared between 0.81–1.21 and 2.18–2.41 ppm as singlets, but the aromatic protons resonated between 6.35–6.88 and 7.25–7.73 ppm as a multiplet. In all complexes (**3a–3d**), –CH-protons of the

p-cymene group were seen as heptets between 2.32 and 2.65 ppm range. NCH₂ resonated as a doublet in the range of 4.32–4.89 and 5.18–5.41 ppm in the ^1H -NMR spectra of **3a–3d**. The obtained complexes (**3a–3d**) display carbene carbon ^{13}C chemical shifts at 187.1, 186.6, 187.7, and 189.2 ppm, respectively. The results are comparable to those seen in other Ru–NHC complexes. Moreover, the Ru complexes **3** were also further characterized by CHN analysis. The results demonstrate good agreement with the calculated percentages of these complexes, with an acceptable range of 0.4% in all cases. The results are comparable to those seen in other Ru–NHC complexes (Molero et al., 1998).

2.3 Biological evaluation

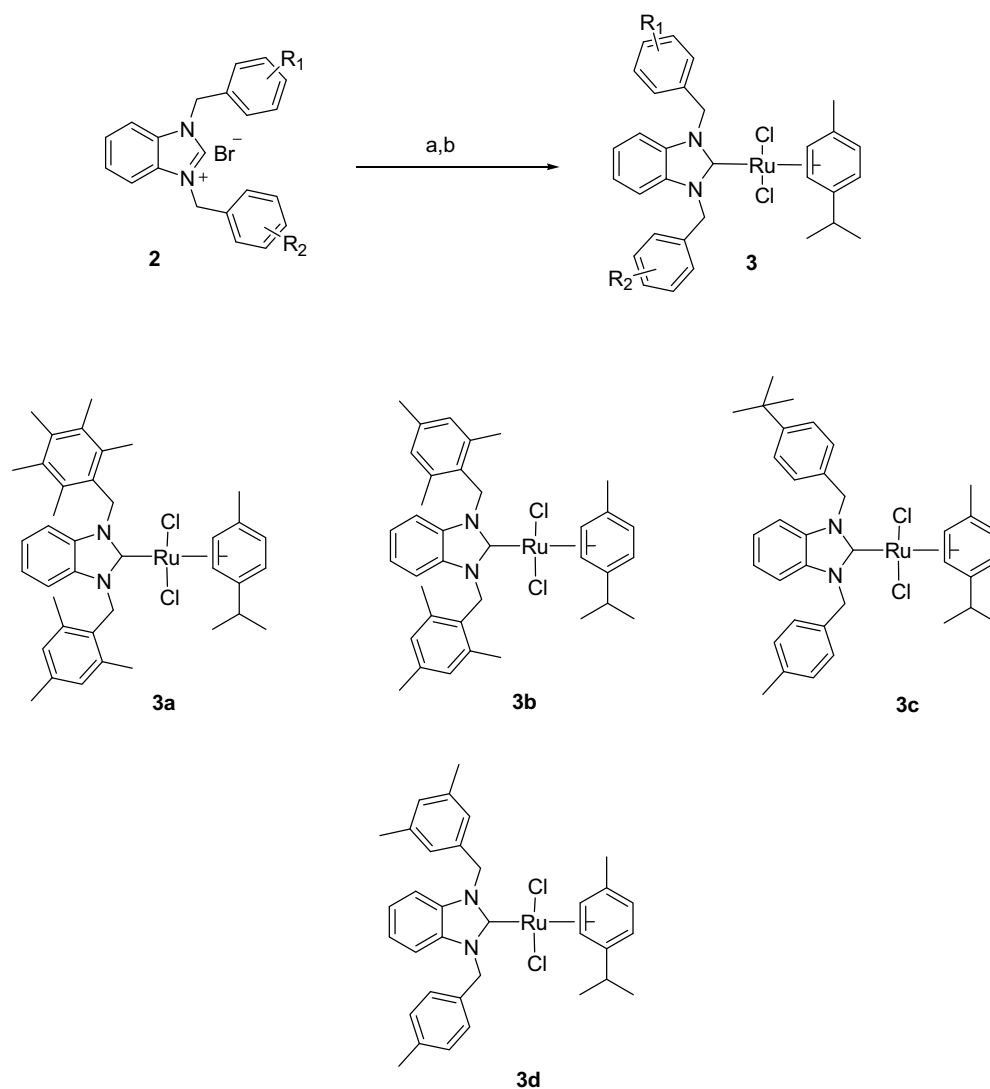
2.3.1 Antimicrobial activity

The obtained compounds were tested *in vitro* for their antibacterial activities using the agar well diffusion method in accordance with earlier studies (Lionetto et al., 2013; Ahmad et al., 2003, and Zolghadri et al., 2019). The minimum inhibition concentration (MIC), which is the lowest concentration of the test sample that completely inhibits the growth of microorganisms, was determined. CMI against *Escherichia coli* (ATCC 25988), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), *Staphylococcus aureus* (ATCC 29213), and methicillin-resistant *Staphylococcus aureus* were measured at a concentration of 100 L. The MIC values of compounds **2–3** against all bacterial strains are shown in Figure 1.

From Figure 1, complexes **3a** and **3c** did not affect the resistance of *E. coli* (ATCC 25988). The **3b** and **3d** complexes were the least effective against the bacteria *P. aeruginosa* (ATCC 27853). Whereas ruthenium(II) complexes (**3a–3d**) demonstrated promising activity comparable to standard, and NHC salts **2** had relatively higher MIC values. The difference in the activity of ligands and complexes may be due to the synergistic effect of ruthenium–ligand bonding that causes the enhancement in lipophilicity of these complexes.

2.3.2 AChE and tyrosinase (TyrE) inhibitory activities

According to previous studies (Zolghadri et al., 2019), the inhibitory activities of ruthenium(II) complexes (**3a–3d**) were studied. The obtained results are represented in IC₅₀ (g·mL^{−1}) units. The most potent inhibitors of TyrE and AChE, according to Figure 2, were complexes **3b** and



Scheme 2: Synthesis of NHC (*p*-Cym)Ru(II)Cl₂ complexes (**3a–3d**): (a) Ag₂O and (b) [(*p*-Cym)RuCl₂]₂.

3d, with IC₅₀ values of 19.88 and 24.95 g·mL^{−1} and 2.52 and 5.06 g·mL^{−1} respectively (Figure 2).

2.3.3 Antioxidant activity

Figure 3 illustrates the strong antioxidant activity of complex **3d**, which is quite similar to BHT. The IC₅₀ values for the other complexes were, in order, 41.78, 32.05, 45.04, and 17.41 g·mL^{−1} (Figure 3).

2.3.4 Antiproliferative activity

The anticancer activities of the synthesized NHC ligands (**2a–2d**) and their corresponding ruthenium(II) complexes

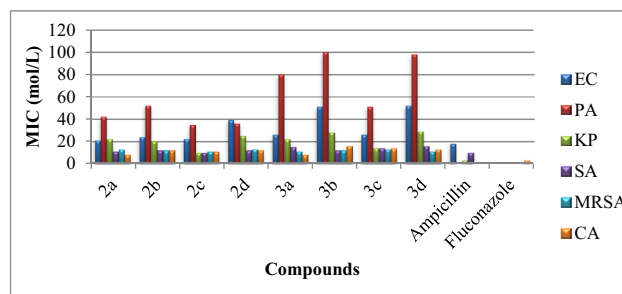


Figure 1: MIC (μmol·L^{−1}) values of salts **2** and their ruthenium(II) complexes (**3a–3d**) against bacterial and fungal strains. (a) **EC**: *E. coli* (ATCC 25988). **PA**: *P. aeruginosa* (ATCC 27853). **KP**: *K. pneumonia* (ATCC 700603). **SA**: *S. aureus* (ATCC 29213). MRSA: Methicillin-resistant *S. aureus* (ATCC 43300). (b) **CA**: *Candida albicans* (ATCC 14053).

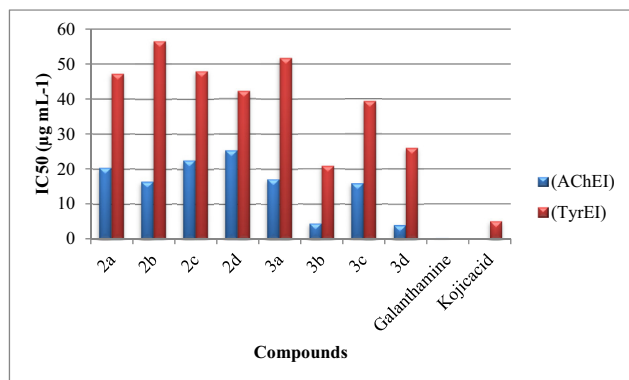


Figure 2: Anti-AChE (AChEI and anti-TyrE (TyrEI) inhibitory activities of the synthesized (NHC) ligands (**2a–2d**) and their respective ruthenium(II) complexes (**3a–3d**) presented by their IC_{50} ($\mu\text{g}\cdot\text{mL}^{-1}$) values.

(**3a–3d**) were studied using the methods for colon carcinoma cell lines (HCT-116) and hepatocellular carcinoma. Compounds **2a**, **2b**, and **3c** had IC_{50} values from 10.45 to 16.45 g in hepatocellular carcinoma cell lines and from 6.65 to 13.45 g in human colon carcinoma cancer cell lines. Moreover, compounds **2c**, **2d**, and **3b** had weak cytotoxic action, with IC_{50} values ranging from 12.27 to 17.22 g in human colon carcinoma cancer cell lines and from 13.25 to 18.32 g in hepatocellular carcinoma cell lines. The results are summarized in Figure 4.

These observations point out that (a) the modification of the steric and electronic properties of NHCs by N-substituents is crucial, (b) the arene type and metal center genus have a significant influence on the antiproliferative activity of complexes, and (c) complexes have properties that facilitate their cellular uptake into cells.

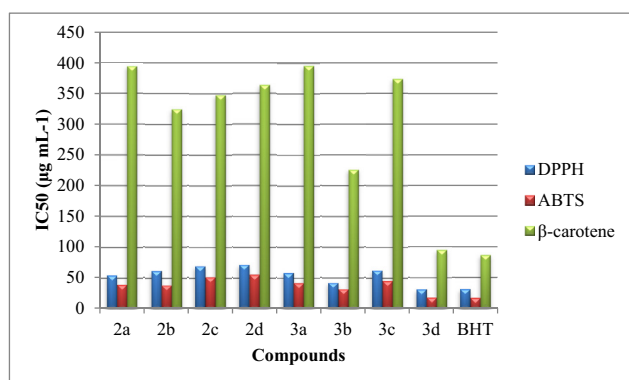


Figure 3: Antioxidant activities of the synthesized NHC ligands (**2a–2d**) and their respective ruthenium(II) complexes (**3a–3d**) assessed by DPPH, ABTS antiradical scavenging power, and β -carotene bleaching test presented by their IC_{50} values.

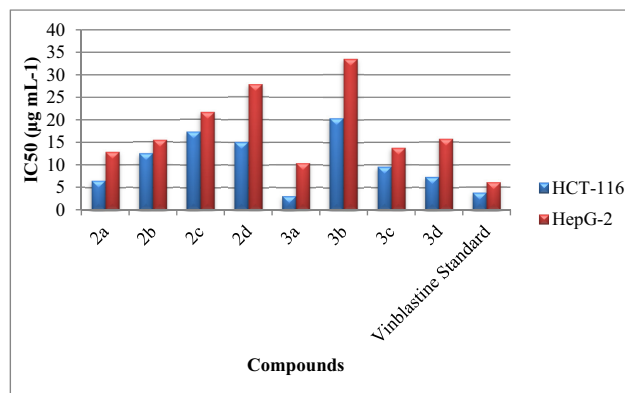


Figure 4: IC_{50} (μg) values for the synthesized NHC ligands (**2a–2d**) and their respective ruthenium(II) complexes (**3a–3d**) and vinblastine against colon carcinoma cells HCT-116 and HepG-2.

3 Conclusions

The target complexes $[\text{RuCl}_2(p\text{-cymene})\text{NHC}]$ (**3a–3d**) were isolated rapidly with good yields following the activation of salts **2a–2d** with silver oxide and then their transmetalation with $[(p\text{-Cym})\text{RuCl}_2]_2$ at room temperature and in dichloromethane. The structures of Ru(II)–NHC complexes **3a–d** and benzimidazolium salts (**2a–2d**) were identified via elemental analysis and from their ^1H and ^{13}C NMR spectra. Enzyme inhibition studies of AChE and TyrE revealed that both complexes **3b** and **3d** have the potent inhibition against AChE and TyrE, respectively. In order to evaluate the antioxidant activity of the synthesized NHC ligands (**2a** tyrosinase **2d**) and their corresponding ruthenium(II) complexes (**3a–3d**) three different methods were used. DPPH (2,2 diphenyl-1-picrylhydrazyl (DPPH)), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and β -carotene linoleic acid bleaching revealed that the **3d** complex showed significant antioxidant activity. As for cancer cell lines (HCT-116) and hepatocellular carcinoma (HepG-2), NHC ligands (**2a–2d**) and their corresponding ruthenium(II) complexes (**3a–3d**) revealed that ruthenium(II) complex **3a** have cytotoxic activity comparable to that of standard vinblastine.

Experimental

General methods

All manipulations were carried out under argon using standard Schlenk line techniques in accordance with our earlier work (Karaca et al., 2021) and (Slimani et al., 2020). Chemicals and solvents were purchased from Sigma-Aldrich Co. (Poole, Dorset, UK). The solvents used were purified

by distillation over the drying agents indicated and were transferred under argon. Melting points were measured in open capillary tubes with an Electrothermal-9200 melting point apparatus. IR spectra were recorded on an ATR unit in the range of 400–4,000 cm^{-1} with PerkinElmer Spectrum 100 Spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded using Bruker Avance AMX and Bruker Avance III spectrometer operating at 400 MHz (^1H NMR) and at 100 MHz (^{13}C NMR) in CDCl_3 with TMS added. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, hept = heptet, and m = multiplet signal. The NMR studies were carried out in high-quality 5 mm NMR tubes. The chemical shifts (δ) are reported in ppm relative to CDCl_3 . Coupling constants (J values) are given in hertz. Elemental analyses were performed by LECO CHNS-932 elementary chemical analyzer

Synthesis of ligands (2a–d)

During a period of 2–3 days, benzimidazolium salt **1** (1 g) and an appropriate amount of benzyl bromide (1 eq) in DMF (2 ml) were agitated at 70°C. Then, the white solid that had formed was agitated for a couple of hours and rinsed with diethyl ether (20 ml). After filtering the reaction mixture through paper, the white solid was dried under vacuum and crystallized with DCM–ether (1:3) for further purification.

1,3-bis(2,3,4,5,6-Pentamethylbenzyl)-1H-benzo[d]imidazol-3-ium bromide (2a)

M.p. = 285°C, yield: 90%, $\nu(\text{CN}) = 1,441 \text{ cm}^{-1}$. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 2.12(s, 9H, CH_3), 2.25(s, 9H, CH_3), 2.20(s, 3H, CH_3), 6.75–7.45(m, HAr). 10.85(s, 1H, H_2). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 16.01(2 CH_3), 19.12(CH_3), 16.9(CH_3), 21.01(CH_3), 21.2(CH_3), 53.1(C_1), 50.2(C_1), 127.7–135.2 (CAr), 143. 2(C_2). Anal. calcd for $\text{C}_{29}\text{H}_{35}\text{BrN}_2$: C, 70.8%; H, 7.1%; N, 5.7%. Found: C, 70.9; H, 7.2; N, 5.8%.

1,3-bis(2,4,6-Trimethylbenzyl)-1H-benzo[d]imidazol-3-ium bromide (2b)

M.p. = 275°C, yield: 95%, $\nu(\text{CN}) = 1,456 \text{ cm}^{-1}$. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 2.15(s, 6H, CH_3), 2.23(s, 9H, CH_3), 2.21(s, 3H, CH_3), 4.40(s, 2H, CH_2), 4.45(s, 2H, CH_2), 6.74–7.45(m, HAr). 10.35 (s, 1H, H_2). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 15.9(CH_3), 18.14(CH_3), 16.3(CH_3), 53.2(C_1), 50.3(C_1), 127.5–135.4 (CAr), 145.

1(C_2). Anal. Calcd for $\text{C}_{27}\text{H}_{31}\text{BrN}_2$: C, 69.9%; H, 6.7%; N, 6.0%. Found: C, 69.9; H, 6.8; N, 6.1%.

1-(4-(tert-butyl)Benzyl)-3-(4-methylbenzyl)-1H-benzo[d]imidazole-3-ium bromide (2c)

M.P. = 285°C, yield: 88%, $\nu(\text{CN}) = 1,445 \text{ cm}^{-1}$. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 2.12(s, 6H, 2 CH_3), 2.11(s, 9H, CH_3), 2.32(m, 1H, CH), 4.56(s, 2H, CH_2), 4.47(s, 2H, CH_2), 6.42–7.57(m, HAr). 11.62 (s, 1H, H_2). ^{13}C NMR(CDCl_3 , 101 MHz) δ (ppm): 15.5(2 CH_3), 18.15(CH_3), 53.1(C_1), 50.4(C_1), 127.6–135.5 (CAr), 144. 2(C_2). Anal. calcd for $\text{C}_{26}\text{H}_{29}\text{BrN}_2$: C, 69.4%; H, 6.5%; N, 6.2%. Found: C, 69.5; H, 6.5; N, 6.3%.

Dimethyl-1,3-(3,5)-dimethyl-4-methylbenzyl)-2,3-dihydro-1H-benzo[d]imidazolium bromide (2d)

Yield: 85%; m.p. = 287°C; FT-IR (KBr) $\nu(\text{CN})(\text{cm}^{-1})$: 1,567; ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 2.12(s, 6H, CH_3), 2.21(s, 3H, CH_3), 4.45(s, 2H, CH_2), 4.48(s, 2H, CH_2), 6.35–7.47(m, HAr). 11.75 (s, 1H, H_2). ^{13}C NMR(CDCl_3 , 101 MHz) δ (ppm): 15.8(CH_3), 17.12(CH_3), 53.1(C_1), 50.4(C_1), 125.5–136.4 (CAr), 144. 7(C_2). Anal. calcd for $\text{C}_{24}\text{H}_{25}\text{BrN}_2$: C, 68.4%; H, 5.9%; N, 6.6%. Found: C, 68.5; H, 5.9; N, 6.7%.

Synthesis of ruthenium NHC complexes (3a–3d)

The ruthenium–NHC complexes were synthesized through transmetalation via silver–NHC complexes (2a–d) without isolation. We reacted 1 eq. of *in situ* silver–NHC complexes and 0.5 eq. of $[\text{RuCl}_2(p\text{-cymene})]_2$ in anhydrous dichloromethane (30 ml). The reaction mixture was stirred for 4 days at room temperature in the dark. Then, the solution was filtered through celite. and the filtrate was concentrated to dryness in a vacuum. The red solid obtained was recrystallized with DCM–diethyl ether or DCM/pentane. The structure of Ru–NHC complexes were determined by IR and NMR analysis.

Dichloro[1-(2,3,4,5,6-pentamethylbenzyl)-3-(2,4,6-trimethylbenzyl)benzimidazol-2-ylidene](p-cymene) ruthenium(II) (3a)

Yield: 85%; m.p. = 224°C. FT-IR(KBr) $\nu(\text{CN})(\text{cm}^{-1}) = 1,410$. ^1H NMR (300 MHz, CDCl_3 , δ (ppm)): 1.12 (d, 6H, 2 CH_3), 2.17 ((s, 3H, CH_3), 1.21 (d, 6H, CH_3), 2.24 (m, 1H, CH_3), 2.13 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.65 (s, 2H, CH_2), 4.52 (s, 2H, CH_2), 6.35–7.35 (m, HAr).

^{13}C NMR (CDCl_3 , 75 MHz) (δ (ppm)): 18.3 (4CH_3), 22.1 (CH_3), 19.5 (CH_3), 20.8 (CH); 22.3(CH_3), 51.7(C_1 , CH_2); 47.8(C_1 , CH_2), 126.3–134.5(CAr). 177.6 (C_2). Anal. calcd for $\text{C}_{39}\text{H}_{48}\text{Cl}_2\text{N}_2\text{Ru}$: C, 65.3%; H, 6.7%; N, 3.9%. Found: C, 65.4%; H, 6.8%; N, 3.9%.

Dichloro[1-(2,4,6-trimethylbenzyl)-3-(2,4,6-trimethylbenzyl)benzimidazol-2-ylidene](*p*-cymene)ruthenium(II) (3b)

Yield: 90%; m.p. = 230°C. FT-IR(KBr) $\nu(\text{CN})(\text{cm}^{-1})$ = 1,418; ^1H NMR (300 MHz, CDCl_3 , δ (ppm)): 2.12 (s, 3H, CH_3), 2.24 ((s, 6H, CH_3), 2.11 (s, 3H, CH_3), 1.12 (d, 6H, CH_3), 2.26 (s, 6H, CH_3), 2.18 (s, 6H, CH_3), 4.5 (s, 2H, CH_2), 4.7 (s, 2H, CH_2), 6.35–7.37 (m, HAr). ^{13}C NMR (CDCl_3 , 75 MHz) (δ (ppm)): 19.1(3CH_3), 20.1(CH_3), 21.1 (CH_3), 21.7 (CH), 22.2 (2CH_3), 21.7 (CH_3), 50.8 (C_1 , CH_2); 47.7 (C_1 , CH_2), 126.2–135.8 (CAr). 189.17 (C_2). Anal. calcd for $\text{C}_{37}\text{H}_{44}\text{Cl}_2\text{N}_2\text{Ru}$: C, 64.5%; H, 6.4%; N, 4.0%. Found: C, 64.6%; H, 6.5%; N, 4.1%.

Dichloro[1-(4-*tert*-butyl) 3-(4-methylbenzyl)benzimidazol-2-ylidene](*p*-cymene)ruthenium(II) (3c)

Yield: 92%; m.p. = 245°C. FT-IR(KBr) $\nu(\text{CN})(\text{cm}^{-1})$ = 1,609. ^1H NMR (300 MHz, CDCl_3 , δ (ppm)): 1.14 (s, 6H, CH_3), 1.26 (s, 9H, CH_3), 2.23 ((s, 3H, CH_3), 2.10(s, 3H, CH_3), 2.63 (d, 1H, CH), 4.61 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 6.37–7.42 (m, HAr). ^{13}C NMR (CDCl_3 , 75 MHz) (δ (ppm)): 31.2 (3CH_3), 22.2 (2CH_3), 21.3 (2CH_3), 32.2 (CH), 51.7 (C_1 , CH_2); 52.4 (C_1 , CH_2), 127.2–143.4 (CAr). 188.95 (C_2). Anal. calcd for $\text{C}_{38}\text{H}_{46}\text{Cl}_2\text{N}_2\text{Ru}$: C, 64.9%; H, 6.6%; N, 3.9%. Found: C, 65.1%; H, 6.7%; N, 4.1%.

Dichloro[1-(3,5-dimethylbenzyl)-3-(4-methylbenzyl)benzimidazol-2-ylidene](*p*-cymene)ruthenium(II) (3d)

Yield: 95%; m.p. = 220°C. FT-IR(KBr) $\nu(\text{CN})(\text{cm}^{-1})$ = 1,409. ^1H NMR (300 MHz, CDCl_3 , δ (ppm)): 1.23 (s, 6H, CH_3), 2.12 (s, 6H), 2.25 ((s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.85 (d, 1H, CH), 4.62 (s, 2H, CH_2), 4.65 (s, 2H, CH_2), 6.39–7.36 (m, HAr). ^{13}C NMR (CDCl_3 , 75 MHz) (δ (ppm)): 21.2 (3CH_3), 22.2 (2CH_3), 21.3 (CH_3), 33.3 (CH), 50.4 (C_1 , CH_2); 52.5 (C_1 , CH_2), 126.5–144.6 (CAr). 182.34 (C_2). Anal. Calcd cor $\text{C}_{34}\text{H}_{38}\text{Cl}_2\text{N}_2\text{Ru}$: C, 63.1%; H, 5.9%; N, 4.3%. Found: C, 63.2%; H, 5.9%; N, 4.4%.

Biological activities

Microorganisms, media, and growth conditions, agar well diffusion method, and MIC were performed according to

the literature work (Vatanabe et al., 2017; Ellman et al., 1961). AChEI activity was measured according to Ellman. The tyrosinase inhibitory activity was determined spectrophotometrically according to the literature. DPPH radical scavenging activity was determined according to Re et al. (1999). ABTS radical scavenging activity was carried out according to Re et al. (1999) protocol. β -Carotene bleaching test was performed according to Pratt's method (Karatas et al., 2016).

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