Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

From Synthesis to Biological Impact of Ru (II) Complexes: Preparation, Characterization, Antimicrobial, Antioxidant scavenging and Enzymatic inhibitory activities

Nasser Amri[1], Yousef E. Mukhrish[1], Rafik Gatri,[2] Nevin Gürbüz.[3,4] ,Ismail Özdemir[3,4] , Khaireddine Dridi [5] and Naceur Hamdi[5]

[1]*Department of Chemistry, Faculty of Science, Jazan University, P.O. Box 2097, Jazan 45142; Saudi Arabia*
[2] *Laboratoire de Synthèse Organique Sélective et Hétérocyclique Évaluation Biologique LR17ES01 Faculté des Sciences de Tunis Faculté des Sciences de Tunis Campus Universitaire 1092 Tunis, Tunisie, Université de Tunis El Manar*
[3]*İnönü University, Catalysis Research and Application Center, 44280-Malatya, Turkey*
[4]*İnönü University, Faculty of Science and Art, Department of Chemistry, 44280 Malatya, Turkey*
[5] *Department of Chemistry, College of Science and Arts at Ar Rass, Qassim University, Ar Rass, 51452, Saudi Arabia*

Abstract

The reaction of $[RuCl_2(p\text{-cymene})]_2$ with in situ prepared Ag-N-heterocyclic carbene (NHC) complexes yields a series of $[RuCl_2(p\text{-cymene})$ (NHC)] complexes **3**. The structures of complexes were established by appropriate spectroscopic methods and elemental analyses. The biological activities of the synthesized ligands and their Ru (II) complexes as acetylcholinesterase, antimicrobial, and antioxidant agents were evaluated. The lowest MICs values were obtained with the two complexes **3b** and **3d**. The enzymatic inhibitory investigation against acetylcholinesterase (AChE) and tyrosinase (TyrE), showed that the two complexes **3b** and **3d** are the most potent inhibitors against (AchE) with an IC_{50} of 2.52 and 5.06 µg mL-1 respectively, and against (TyrE) with an IC_{50} of 19.88 and 24.95 µg mL-1 respectively. Additionally, DPPH (2,2-diphenyl-1-picrylhydrazyl) has been tested for its ability to scavenge hydrogen peroxide and free radicals. According to our results, these compounds exhibit excellent radical scavenging properties

Keywords: Strucutre analysis , Catalysts , Catalysis , Biological activity, N-Heterocyclic carbene, benzimidazolium salts, ruthenium complexes, silver complexes.

1. Introduction

Nowadays, the use of organometallic and inorganic compounds is very common in contemporary medication (1-2). N-heterocyclic carbene (NHC) complexes are the new promising members of organometallic complexes for drug formulation (3-7). The first study on the biological activities of NHC complexes was reported by Çetinkaya (8-11) and co-workers. A number of research groups, including ours, have synthesized functionalized NHC complexes and explored their biological properties for this purpose (12-16). Some studies showed that the main target of palladium NHCs in cancer cells is DNA similar to cisplatin (17). In this regard, ruthenium (II/III) tpy complexes have been extensively investigated as anticancer and antimicrobial drugs as well as DNA binding agents.(18-21) In particular, in the design of innovative anticancer drugs, ruthenium tpy complexes have special consideration and have been evaluated against different cancer cell lines as promising alternates for the well-known diamine-dichloroplatinum (II) (cisplatin) and its derivatives.(22) Ruthenium accesses +2 and +3 oxidation states at physiological conditions and can bind to proteins, nucleic acids, sulfur, or oxygen-containing compounds in the cells (23-25). 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 make 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 also be effective in the cells resistant to cisplatin. In addition, ruthenium complexes have emerged as a new and very interesting class of biologically active

*Corresponding author e-mail: hamdi_naceur@yahoo.fr; (Naceur HAMDI)
Receive Date: 19 February 2022, Revise Date: 01 March 2023, Accept Date: 03 April 2023.
DOI: 10.21608/EJCHEM.2023.122780.5495

agents. Our research group is also studying metal complexes with different biological activites (26-28). We are currently researching novel functional NHC ligands, which provide a favorable environment for the development and valorization of metal compounds. In this paper, we have synthesized and characterized a new series of Ru(II)-NHC complexes containing benzimidazole. The structures of the new compounds were characterized by different spectroscopic and analytical methods. Furthemore, their different biological activities such as antimicrobial activity, antioxidant activity and enzymatic inhibitory were studied.

2. Experimental

2.1. General methods

All manipulations were carried out under argon using standard Schlenk line techniques. 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 measures in open capillary tubes with an Electrothermal-9200 melting points apparatus. IR spectra were recorded on ATR unit in the range of 400-4000 cm^{-1} with Perkin Elmer Spectrum 100 Spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Avance AMX and Bruker Avance III spectrometer operating at 400 MHz (^{1}H 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 5mm NMR tubes. The chemical shifts (d) 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.

2.2. Synthesis of ligands (2a-d)

A mixture of benzimidazolium salt **1** (1 g) and the corresponding benzyl bromide (1eq) in DMF (2 ml) was stirred at 70°C for 2-3 days. After that time, the white solid formed was washed with diethyl ether (20 ml) and stirred for a couple of hours. Then the reaction mixture was filtered through filter paper and the white solid was dried under vaccum then crystallized with DCM-ether (1:3) for further purification

5-methyl-1,3-bis(2,3,4,5-pentamethylbenzyl)-1H-benzo[d]imidazol-3-ium bromide 2a
m.p. 312°C. Yield (95%). ν(CN)= 1441cm^{-1} . ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 2.17(s, 9H, CH_3), 2.28(s, 9H, $3CH_3$), 2.23(s, 3H, CH_3), 2.23(s, 3H, CH_3), 2.35(s, 3H, CH_3),6.92-7.40 (m, Harom). 9.85 (s, 1H, H_2).^{13}CNMR($CDCl_3$, 101MHz) δ (ppm): 16.01($2CH_3$), 19.12($3CH_3$), 16.9(CH_3), 21.01(CH_3), 21.2(CH_3), 53.1(C_1), 50.2($C_{1'}$), 127.7-135.2 (Carom), 141. 5(C_2).Anal. Calcd for $C_{29}H_{35}BrN_2$: C, 70.87%; H, 7.18%; N, 5.70%. Found: C, 70.8; H, 7.1; N, 5.8%.

5-methyl-1,3-bis(2,6-dimethylbenzyl)-1H-benzo[d]imidazol-3-ium bromide 2b
m.p. 215°C. Yield (90%). ν(CN)= 1456cm-1 . ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 2.15(s, 6H, $2CH_3$), 2.23(s, 9H, $3CH_3$), 2.21(s, 3H, CH_3), 4.40(s, 2H, CH_2), 4.45(s, 2H, CH_2), 6.74-7.45(m, Harom). 10.34 (s, 1H, H_2).^{13}CNMR($CDCl_3$, 101MHz) δ (ppm): 15.9($2CH_3$), 18.14($3CH_3$), 16.3(CH_3), 53.2(C_1), 50.3($C_{1'}$), 127.5-135.4 (Carom), 141. 3(C_2). Anal. Calcd for $C_{27}H_{31}BrN_2$: C, 69.97%; H, 6.74%; N, 6.04%. Found: C, 69.9; H, 6.8; N, 6.1%.

5-methyl-1,3- ((4-(tert-butyl)-4-methyl-benzyl)-1H-benzo[d]imidazol-3-ium bromide 2c
m.p. 293°C. Yield (86%). ν(CN)= 1428cm-1 . ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 2.15(s, 6H, $2CH_3$), 2.22(s, 9H, $3CH_3$), 2.32(m,1H,CH), 4.51(s,2H, CH_2) , 4.45(s,2H, CH_2) ,6.36-7.47(m, Harom). 11.83 (s, 1H, H_2). ^{13}CNMR($CDCl_3$, 101MHz) δ (ppm): 15.5($2CH_3$), 18.15($3CH_3$), 53.1(C_1), 50.4($C_{1'}$), 127.6-135.5 (Carom), 141. 7(C_2). Anal. Calcd for $C_{27}H_{31}BrN_2$: C, 69.97%; H, 6.74%; N, 6.04%. Found: C, 69.9; H, 6.8; N, 6.1%.

5-methyl-1,3-(3,5)-dimethyl-4-methylbenzyl)-2,3-dihydro-1H-benzo[d]imidazolium bromide (2d)
Yield: 92%; M.p.= 235°C; FT-IR (KBr) (cm^{-1}): = 1567 (C=N); 1359 (C-N) cm^{-1} ; ^{1}H NMR ($CDCl_3$, 400MHz) δ (ppm) 2.12(s, 6H, $2CH_3$), 2.231(s, 6H, $2CH_3$), 4.43(s, 2H, CH_2), 4.47(s, 2H, CH_2), 6.35-7.47(m, Harom). 11.58 (s, 1H, H_2). ^{13}CNMR($CDCl_3$, 101MHz) δ (ppm): 15.7($2CH_3$), 18.12($2CH_3$), 53.1(C_1), 50.4($C_{1'}$), 125.5-136.4 (Carom), 141. 5(C_2). Anal. Calcd for $C_{27}H_{31}BrN_2$: C, 68.96 %; H, 6.25%; N, 6.43%. Found: C, 68.9; H, 6.2; N, 6.15%

2.3. Synthesis of ruthenium N-heterocyclic Carbene complexes (3a-3d) :

Ag_2O (0.54 mmol) was added to a solution of benzimidazole salts **2** (1.08 mmol) in dichloromethane (25 mL) under an atmosphere of argon. The mixture was stirred for 24 h at room temperature, covered with aluminum foil, and then filtered through celite to remove the formed AgBr. $[RuCl_2(pcymene)]_2$ (0.5 mmol) was added to the colorless solution, and the reaction mixture was stirred for 24 h at room

temperature. The resulting mixture was filtered through celite, and the solvent was removed under vacuum to afford the product. The crude product was recrystallized from dichloromethane:diethyl ether (1:2) at room temperature. The orangebrown crystals were filtered off, washed with diethyl ether (3 × 10 mL) and dried under vacuum.The structure of Ru-NHC complexes were determined by IR and NMR analysis

5-methyl-[1,3-(2,3,4,5-pentamethyl-2,4,6-trimethyl)-benzimidazol-2-ylidene](p-cymene) ruthenium(II) chloride, (3a)

Yield: (80 %); m.p= 204°C. FT-IR(KBr)ν(CN)(cm^{-1})=1404.^{1}HNMR (300 MHz, $CDCl_3$, δ(ppm)): 1.2 (d,6H,CH_3),2.28((s,3H,CH_3), 1.2 (d,6H,CH_3),2.36 (m,1H,CH_3), 2.1 (s,3H,CH_3), 2.28 (s,9H,CH_3), 2.18 (s,12H,4CH_3), 4.6 (s,2H,CH_2), 4.5 (s,2H,CH_2),6.35-7.35(m,Harom).^{13}C NMR ($CDCl_3$, 75 MHz) (δ (ppm)):19.4(4CH_3), 21.2 (CH_3), 21.3 (CH_3), 21.9 (CH) 23.2(2CH_3) ,21.9 (3CH_3), 50.9 ($C_{1'}$, CH_2); 47.9 (C_1, CH_2), 126.1-135.6(Carom). 189.2 (C_2). Anal. Calcd for $C_{41}H_{53}RuN_2Cl_2$: C, 65.35%; H, 6.75%; N, 3.91%. Found: C, 65.4; H, 6.7; N, 3.9%.

5-methyl-[1,3-(2,6-dimethyl-2,4,6-trimethyl)-benzimidazol-2-ylidene](p-cymene) ruthenium(II) chloride, (3b)

Yield: (88 %); m.p=184°C. FT-IR(KBr)ν(CN)(cm^{-1})= 1418 ; ^{1}HNMR (300 MHz, $CDCl_3$, δ(ppm)): 2.15 (s,3H,CH_3),2.28((s,6H,2CH_3), 2.12(s,3H,CH_3),1.1 (d,6H,2CH_3),2.29 (s,6H,2CH_3), 2.29(s,6H,2CH_3), 2.28 (s,9H,CH_3), 2.18 (s,12H,4CH_3), 4.6 (s,2H,CH_2), 4.5 (s,2H,CH_2),6.34-7.36(m,Harom).^{13}C NMR ($CDCl_3$, 75 MHz) (δ (ppm)):19.2(3CH_3), 21.1 (CH_3), 21.2(CH_3), 21.8 (CH) ,22.1(2CH_3) ,21.7 (2CH_3), 50.8 ($C_{1'}$, CH_2); 47.7 (C_1, CH_2), 126.2-135.8(Carom). 187.8 (C_2). Anal. Calcd for $C_{37}H_{44}Cl_2N_2Ru$: C, 64.52 %; H, 6.44 %; N, 4.07 %. Found: C, 64.5; H, 6.4; N, 4.1%.

5-methyl-[1,3-(4-(tert-butyl) -4-methyl) -benzimidazol-2-ylidene] (p-cymene) ruthenium (II) chloride, (3c)

Yield: (87 %); m.p=224°C. FT-IR(KBr)ν(CN)(cm–1)= 1609 (C-N). ^{1}HNMR (300 MHz, $CDCl_3$, δ(ppm)):1.21(s,6H,2CH_3), 1.31 (s,9H,3CH_3),2.28((s,3H, CH_3), 2.11(s,3H,CH_3), 2.85(d,1H,CH), 4.61 (s,2H,CH_2), 4.62 (s,2H,CH_2),6.35-7.39(m,Harom).^{13}C NMR ($CDCl_3$, 75 MHz) (δ (ppm)):31.2(3CH_3), 23.1 (2CH_3), 21.1(2CH_3), 21.1 (CH_3) , 33.1 (CH), 51.8 ($C_{1'}$, CH_2); 52.7 (C_1, CH_2), 126.3-142.8(Carom). 188.9 (C_2). Anal. Calcd for $C_{37}H_{44}Cl_2N_2Ru$: C, 64.52 %; H, 6.44 %; N, 4.07 %. Found: C, 64.5; H, 6.4; N, 4.1%.

5-methyl-[1,3-(3,5)dimethyl-4-methyl)-benzimidazol-2-ylidene](p-cymene) ruthenium(II) chloride, (3d)

Yield: (90 %); m.p=210°C. FT-IR(KBr)ν(CN)(cm–1)= 1409 (C-N). ^{1}HNMR (300 MHz, $CDCl_3$, δ(ppm)):1.20(s,6H,2CH_3), 2.13 (s,6H,2CH_3),2.28((s,3H, CH_3), 2.28(s,3H,CH_3), 2.31(s,3H,CH_3), 2.86(d,1H,CH), 4.62 (s,2H,CH_2), 4.63 (s,2H,CH_2),6.37-7.35(m,Harom).^{13}C NMR ($CDCl_3$, 75 MHz) (δ (ppm)):21.3(3CH_3), 23.1 (2CH_3), 21.4(CH_3), 19.1 (CH_3) , 33.2 (CH), 50.3 ($C_{1'}$, CH_2); 52.6 (C_1, CH_2), 126.4-143.5(Carom). 189.0 (C_2). Anal. Calcd for $C_{35}H_{38}Cl_2N_2Ru$: C, 63.82 %; H, 5.82 %; N, 4.25 %. Found: C, 63.8; H, 5.8; N, 4.2%.

2.4. Biological activities

2.4.1. Antibacterial activity:

Bacterial strains, media and growth conditions

Bacteria strains used as indicator microorganisms for the antibacterial activity assays were: *Micrococcus luteus (M. luteus)* LB 14110, *Staphylococcus aureus* (S. aureus) ATCC6538, *Listeria monocytogenes* (L. monocytogenes) ATCC 19117, *Salmonella Typhimurium* (S. Typhimurium) ATCC 14028, and *Pseudomonas aeruginosa* (P. aeruginosa) ATCC 49189 were obtained from International Culture Collections (ATCC). These bacterial strains were grown overnight in Luria-Bertani (LB) agar medium (g/L): peptone 10; yeast extract 5 and NaCl 5 at pH 7.2 under aerobic conditions and constant agitation (200 rpm) at 30°C for *M. luteus* LB14110 and *L. monocytogenes* ATCC 19117 and at 37 °C for *S. aureus* ATCC 6538, *S. Typhimurium* ATCC 14028 and *P. aeruginosa* ATCC49189, and then diluted 1:100 in LB media and incubated for 5 h under constant agitation (200 rpm) at the appropriate temperature.

Agar well diffusion method

Agar well diffusion method was employed for the determination of the antimicrobials activity of the synthesized compounds according to Güven et al. (29) with some modifications. Briefly, the synthesized compounds are allowed to diffuse out into the appropriate agar medium (LB agar medium) and interact in a plate freshly seeded with a suspension of the indicators microorganisms (0.1 ml of 108 cells per ml). The plate was incubated at the appropriate temperature after staying at 4°C for 2 h. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The antibacterial activity was assayed by measuring in millimeters the diameter of the inhibition zone formed around the well. All tests are assayed in triplicate and expressed as the average ± standard deviation of the measurements.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the synthesized compounds was determined by NCCLS

guideline M7-A6 and M38-P (National Committee for clinical laboratory standard, Wayne 1998) (30). The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 µl. The synthesized compounds (20 mg/mL) were properly prepared in solution of dimethylsulfoxide (DMSO)/water (1/9; v/v). The inhibitory activity of each synthesized compound was transferred to each well to obtain a twofold serial dilution of the original sample and visible growth after incubation. As an indicator of microorganism growth, 25 µl of Thiazolyl Blue Tetrazolium Bromide (MTT), indicator solution (0.5 mg/ml) dissolved in sterile water was added to the wells and incubated at room temperature for 30 min. This determination was done in triplicate and the obtained results were very similar. The reported value is the average of the three tests.

2.4.2. Enzymatic inhibitory

Acetylcholinesterase inhibitory (AChEI).

(AChEI) activity was measured according to spectrophotometric method of Electric eel AChE described by Ellman et *al*.(31) Acetylthiocholine iodide (ATCI) was employed as substrate of the reaction and 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was used for the measurement of the antiacetylcholinesterase activity.

Antityrosinase activity

The tyrosinase inhibitory activity was determined spectrophotometrically according to Rangkadilok et *al*.,(32) using L-tyrosine as substrate and carried out in a 96-well microplate

Antioxidant activity

Antioxidant activity was expressed as IC_{50} (the concentration that causes 50 % of the effect). The antioxidant activity of the synthesized compounds was assessed by three different techniques which are: the 2.2-diphenyl-1-picrylhydrazyl (DPPH), the 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging and the □-carotene linoleic acid bleaching assay. Butylated hydroxytoluene (BHT), known as a strong antioxidant compound, was used as a control.

(DPPH) radical scavenging activity

Briefly, synthesized compounds were dissolved in dimethylsulfoxide (DMSO)/water (1/9; v/v) and diluted with ultrapure water at different concentrations (1, 0.5, 0.250, 0.125, 0.0625, 0.03125 mg mL^{-1}). Then, 500 µL of a 4% (w/v) solution of DPPH radical in ethanol was mixed with 500 µL of samples. The mixture was incubated for 30 min in the dark at room temperature.(33) The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank. The percentage of antiradical activity (% ArA) had been calculated as follows :

% ArA = [(absorbance of control - absorbance of test sample) / absorbance of Control] X 100

(ABTS) radical scavenging activity protocol.

ABTS radical scavenging activity. This determination was carried out according to Re et al. (34 protocol. Twenty mL of solution of synthesized compounds or control were mixed with 18 mL of ABTS solution; the whole was mixed vigorously for 30 s. A control consisting of ethanol and ultrapure water was prepared (5:5) v/v to which the previous solution was added. White is ethanol and ultrapure water (5:5) v/v. Samples and controls were incubated for 6 min in the dark, then the OD was measured at 734 nm. The calculation is done according to the following formula: Inhibition (%) = [(Absorbance control-Absorbance sample)/Absorbance Control] X 100 : absorbance of the control, Ae: absorbance of the sample

3. Results and discussion

3.1. Preparation of benzimidazolium salts 2a-d

The benzimidazoles salts (**2a-d**) were prepared via the two step N-alkylation process as depicted in **Figure 1**. Compound **1** was obtained by N-alkylation of 5-methyl benzimidazole with aryl bromide in the presence of KOH in EtOH at 80°C for 8 h. The benzimidazolium salts (**2a-d**) were prepared by reacting compound (1) with various aryl bromide in DMF at 70°C for 24 h (**Figure 1**). The reaction has been monitored following thin layer chromatography, and after this time the formation of salts (**2a-d**), has been observed for every target compound. The benzimidazolium salts (**2a-d**) were air- and moisture stable both in the solid state and in solution. The FTIR spectroscopy, ^{1} H- and ^{13}C{1H} NMR spectroscopy, and elemental analysis data of the title compounds confirm the proposed structures.

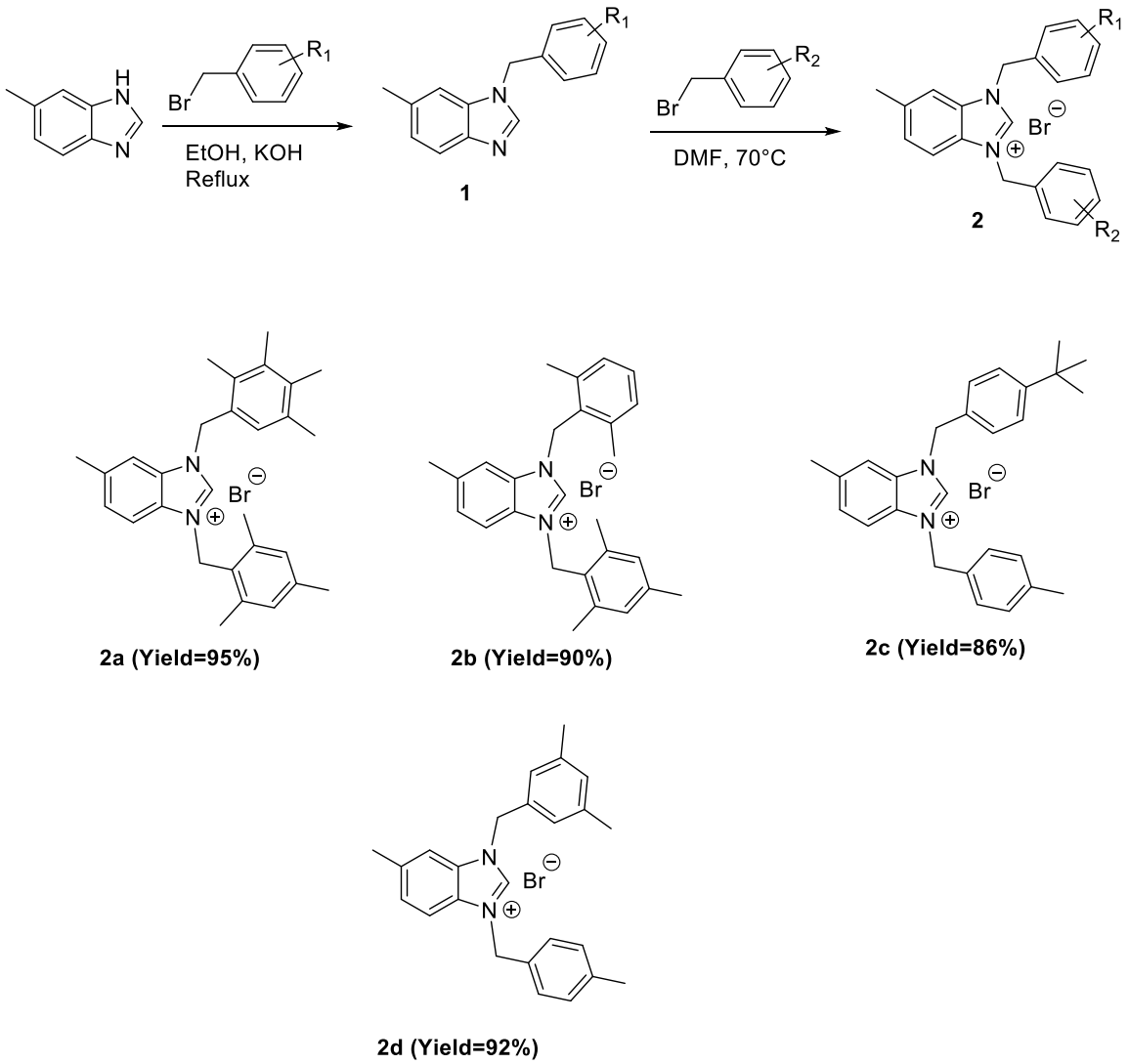

Figure 1 : General preparation and strucutres of benzimidazolium salts (**2a-2d**)

The ^{1}H NMR spectra of precursors **2a–2d** show a characteristic downfield shift in the range δ 9.85–11.83 ppm for the NCHN proton, attributable to the positive charge of the molecule. (35) The ^{1}H NMR spectra of the benzimidazolium salts further supported the assigned structures; the resonances for C(2)– H were observed as sharp singlets at 9.85, 10.34,11.83 and 11.58 ppm, respectively, for **2a–c**. ^{13}C NMR chemical shifts were consistent with the proposed structures; the imino carbon are typical singlets in the 1H-decoupled mode at 141.5, 143.3 , 141.7 and 141.5 ppm, respectively, for benzimidazolium bromides **2a–d**. In the ^{13}C NMR spectra, similarly, aromatic rings were observed in the region 113.38–152.82 ppm, while the aliphatic region evidenced a set of peaks in the range 20.76–35.18 ppm, which were assigned to the resonance of aliphatic carbon nuclei. These values are in good agreement with the previously reported results. (36)

Preparation of ruthenium-carbene complexes 3a-3d

Ru(II)-NHC complexes (**3a-d**) are prepared using a variety of methods. One of the most useful approaches in this regard is transmetalation with Ag-NHC complexes. The use of silver-carbene complexes as a carbene transfer agent avoids complicated working difficulties. (37) The new [$RuCl_2$(p-cymene)(NHC)] complexes (**3a-3d**) were prepared using a two-step process by transmetalling the respective silver NHC derivatives without isolation, the silver-NHC complexes were used in situ. Then, adding [$RuCl_2$(p-cymene)]$_2$ to the mixture gave orange-brown complexes with good yields (80–90%). Ruthenium carbene complexes (**3a-3d**) are soluble in solvents such as chloroform, dichloromethane; and tetrahydrofuran, but not in nonpolar solvents. Synthesis and structures of Ru(II)-NHCs are given in

Figure 2. The structures of complexes **3a-3d** were established by ^{1}H NMR, ^{13}C NMR and IR spectroscopic techniques and elemental analyses

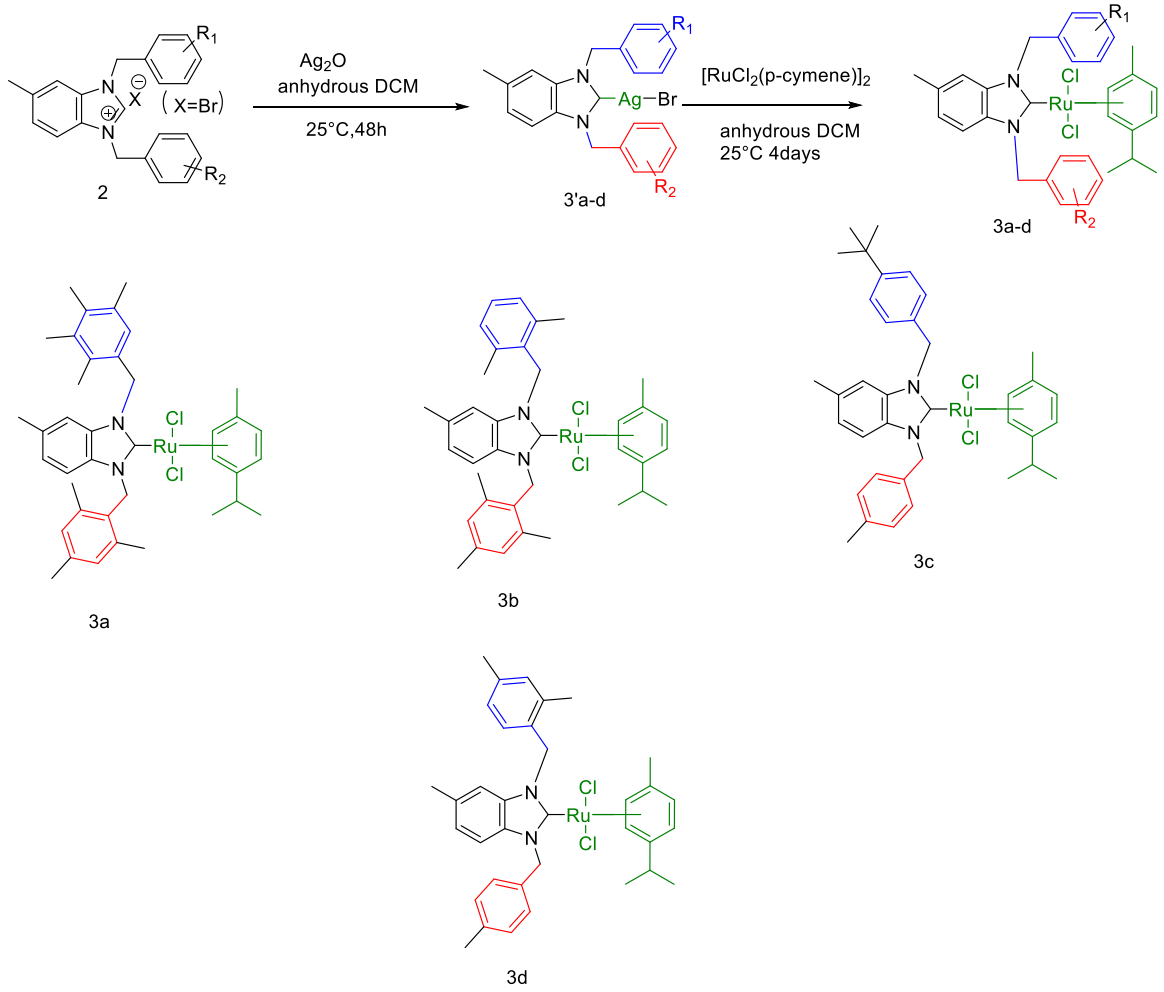

Figure 2: General preparation of ruthenium N-Heterocyclic carbene complexes (**3a-3d**)

The aromatic protons of complexes **3a-3d** appeared between 6.34-7.36 ppm as a multiplet while methylic protons appeared between 1.2 and 2.31 ppm as singlets. –CH-Protons of the p-cymene group on all complexes (**3a-3d**) are seen as heptets in the range from 2.64 to 2.86 ppm. In the ^{1}H-NMR spectra of (**3a-3d**), (NCH_2) resonated at between 4.5-4.6 ppm as a doublet. The complexes exhibit ^{13}C chemical shifts of carbene carbon at 189.2, 187.8, 188.9 and 189.0 ppm, respectively. The data are close to those reported for other Ru-NHC complexes (38) which confirmed the transmetallation between silver and ruthenium. In order to explain the structures of our complexes in more detail, a suitable crystal of the Ru complex for single-crystal Xray diffraction study was tried to be obtained many times by solvent diffusion method using various solvent systems such as CH_2Cl_2/Et_2O, $EtOH/Et_2O$, and etc. But, despite all efforts, a suitable single crystal for X-ray analysis could not be obtained from the Ru-complexes. In addition, the benzimidazoles salts **2** and their Ru-complexes **3** are further characterized by CHN microelemental analyses and the data obtained is in well agreement with the calculated percentages of these elements with an acceptable limit of variation with ±0.4% in all the cases. The elemental analysis data of the complexes confirmed their expected stoichiometry and the percentage yield varied from 43%–63%.

3.2. Biological evaluation

3.2.1. Antimicrobial activity

The synthesized compounds were examined in vitro for their antimicrobial activity using the agar well diffusion method. Six indicator microorganisms were used which are S. aureus and L. monocytogenes, *E.*

coli, *P. aeruginosa* and *S. typhimurium* and the fungus *C. albicans*. *S. aureus* is an eminent human pathogen that can colonize the human host and cause severe life-threatening illnesses. (39) L. monocytogenes, a facultative anaerobic bacterium, is a species of pathogenic bacteria that causes the infection of listeriosis. It can grow and reproduce inside the host's cells and is one of the most virulent foodborne pathogens.(40) 20 to 30% of foodborne listeriosis infections in high-risk individuals may be fatal.(40) P. aeruginosa, is an opportunistic gram-negative bacillus that is responsible for a wide variety of infections in humans ranging from relatively uncomplicated urinary tract infections to severe and life-threatening infections, including neonatal sepsis and chronic lung infections in patients with cystic fibrosis.(41) S. typhimurium the causative agent of typhoid fever is a major public health threat and an estimated 26.9 million cases resulting in approximately 217,000 deaths occur annually by this pathogenic bacterium.(42) C. albicans is a ubiquitous pathobiotic microorganism, a member of commensal flora, which can cause infections in both healthy and immunocompromised patients. Candidiasis caused by C. albicans may be superficial or profound. (43) Despite the fact that common C. albicans infections are easily treatable, systemic infection, frequently of nosocomial nature, have a high mortality rate. (44)

The antimicrobial activity of the synthesized compounds N-Heterocyclic carbene (NHC) ligands (**2a-2d**) and their respective ruthenium(II) complexes (**3a-3d**) against the five indicator microorganisms was shown in **Figure 3**. Complexes **3b** and **3d** presented inhibitory activity against all tested indicator microorganisms and the highest inhibition of growth was obtained with these two synthesized compounds (**Figure 3**). Complex **3c** causes inhibition zones of 22, 23, 21 and 25 mm against S. aureus, L. monocytogenes, E. coli, P. aeruginosa, S. typhimurium and C. albicans respectively (Figure 3). Synthesized compound **3a** causes inhibition zones of 35, 23, 24 and 22 mm against S. aureus,

[a] Zone of bacterial inhibition measured in mm

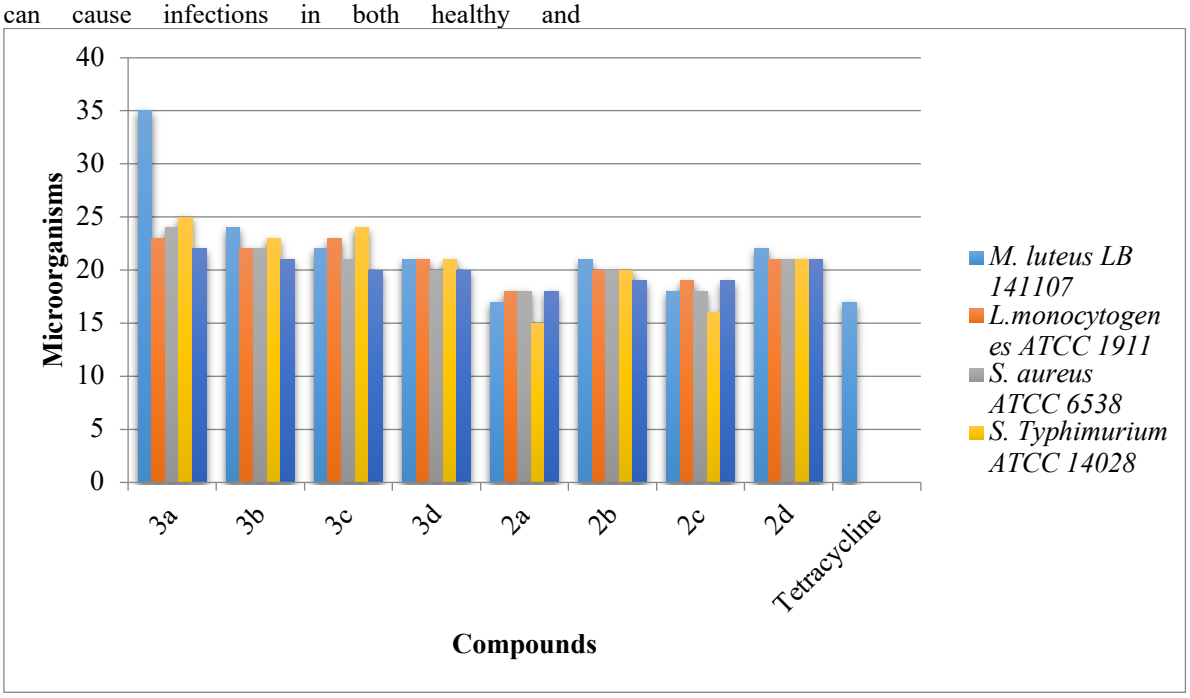

Figure 3. Antimicrobial activity of the synthesized N-Heterocyclic carbene (NHC) ligands (**2a-2d**) and their respective ruthenium(II) complexes (**3a-3d**) against the five tested indicator microorganisms. The diameters of inhibition zones were reported in mm.

Minimum inhibitory concentration (MIC) of the synthesized compounds N-Heterocyclic carbene (NHC) ligands (**2a-2d**) and their respective ruthenium(II) complexes (**3a-3d**) and the standards ampicillin and kanamycin were assessed by micro-dilution method against three indicator microorganisms: *Listeria monocytogenes* ATCC 1911, *Staphylococcus aureus* ATCC 6538 and *Salmonella typhimurium* ATCC 14028. As shown in **Figure 4.** The diameters of inhibition zones were reported in mm. **Figure 4**, the MICs values range from 0.1552 to 0.1643 µg mL^{-1} against *L. monocytogenes*, from 0.1463 to 0.1612 µg mL^{-1} against S. aureus and S. typhimurium from 0.1561 to 0.3136 µg mL^{-1}. For the synthesized

compound **3b**, MIC value was 0.1562, 0.1542 and 0.1572 µg mL-1 against *Listeria monocytogenes* ATCC 1911, *Staphylococcus aureus* ATCC 6538 and *Salmonella typhimurium* ATCC 14028. (**Figure 4**).

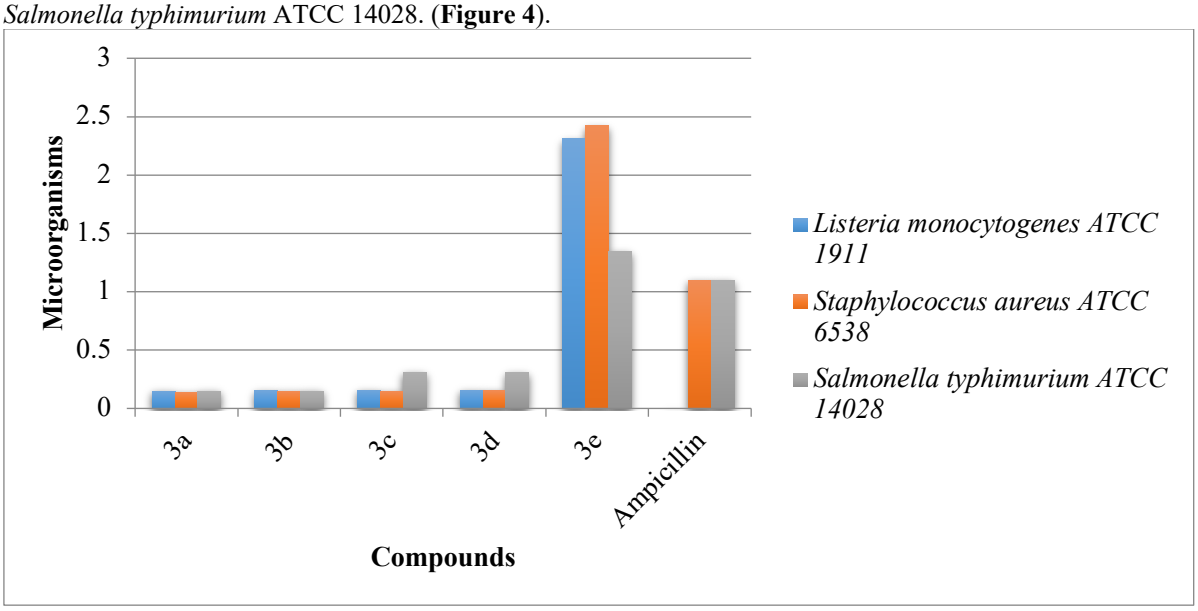

Figure 4. Minimum Inhibitory Concentration (MIC) of synthesized ruthenium(II) complexes (**3a-3d**) against *L. monocytogenes*, *S. aureus* and *S. typhimurium*

3.2.2. Enzymatic inhibitory, (AChE) and (TyrE) inhibitory activity

AChE belongs to the family of cholinesterases (ChEs), which are specialized carboxylic ester hydrolases that break down esters of choline. The Cholinesterase class includes AChE which hydrolyzes the neurotransmitter acetylcholine and pseudocholinesterase or butyrylcholinesterase (BChE) which utilizes butyrylcholine as substrate. AChE is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system. (45). Other possible therapeutic applications in the treatment of Parkinson's disease. (46-47) Tyrosinase (TyrE) is a multi-copper enzyme that is widely distributed in different organisms and plays an important role in melanogenesis and enzymatic browning. Therefore, its inhibitors can be attractive in cosmetics and medicinal industries as depigmentation agents and also in food and agriculture industries as antibrowning compounds. (48) Tyrosinase is the main enzyme recognised as responsible for melanogenesis in mammals. (49) This fact, encouraged researchers and scientists to focus on the identification, isolation, synthesis and characterisation of new potent tyrosinase inhibitors for the medicinal industry. (AChE) and (TyrE) inhibitory activities were expressed in IC_{50} (µg mL-1). IC_{50} (half maximal inhibitory concentration) is a quantitative measure that indicates how much of a particular inhibitory substance is needed to inhibit, in vitro, a given biological process or biological component. The results presented in Figure 5, show that the complexes **3b** and **3d**, are the most potent inhibitors against AchE with an IC_{50} of 2.52 and 5.06 µg mL-1 respectively, and against TyrE with an IC_{50} of 19.88 and 24.95 µg mL-1 respectively. However, it should be noted that IC_{50} values of complexes **3b** and **3d**, against the two enzymes (AchE) and (TyrE) are very higher than those of the two used standards. In fact, the IC_{50} of the Galanthamine against (AchE) is 0.25 µg mL-1 (ten and twenty times lower than that of the complexes **3b** and **3d** respectively), and the IC_{50} of the kojic acid against (TyrE) is 5.05 µg mL-1 (four and five times lower than that of the complexes **3b** and **3d** respectively).

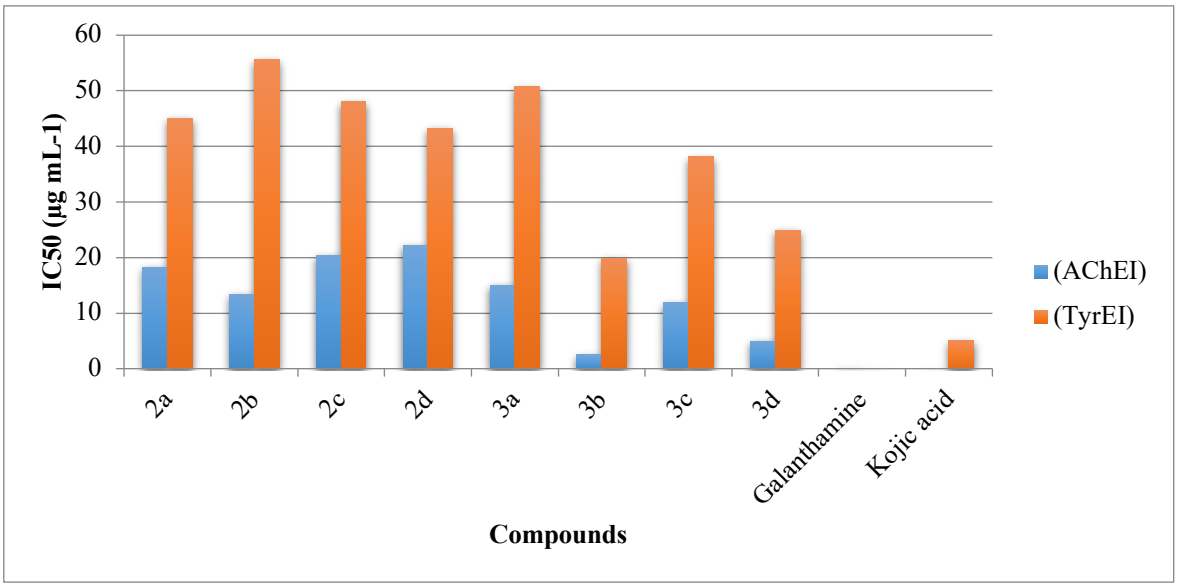

Figure 5. Anti-acetylcholinesterase (AChEI) and anti-tyrosinase (TyrEI) inhibitory activity of the synthesized N-Heterocyclic carbene (NHC) ligands (**2a-2d**) and their respective ruthenium(II) complexes (**3a-3d**) presented by their IC_{50} (µg mL^{-1}).

3.2.3. 4.3. Antioxydant Activity

The antioxidant activity of the synthesized compounds was assessed by two different techniques: the 2.2-diphenyl-1-picrylhydrazyl (DPPH) and the 2.2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging. Results were expressed as IC_{50} values in µg mL^{-1}. IC_{50} (the concentration required to obtain a 50% antioxidant effect), is a typically employed parameter to express the antioxidant capacity and to compare the activity of different Compounds. As shown in Figure 6, complex **3d** has recorded an important antioxidant activity very similar to that of the standard BHT. In fact, the IC_{50} of the others complexes were 48.07, 47,17 and 46.90 µg mL^{-1} for DPPH.

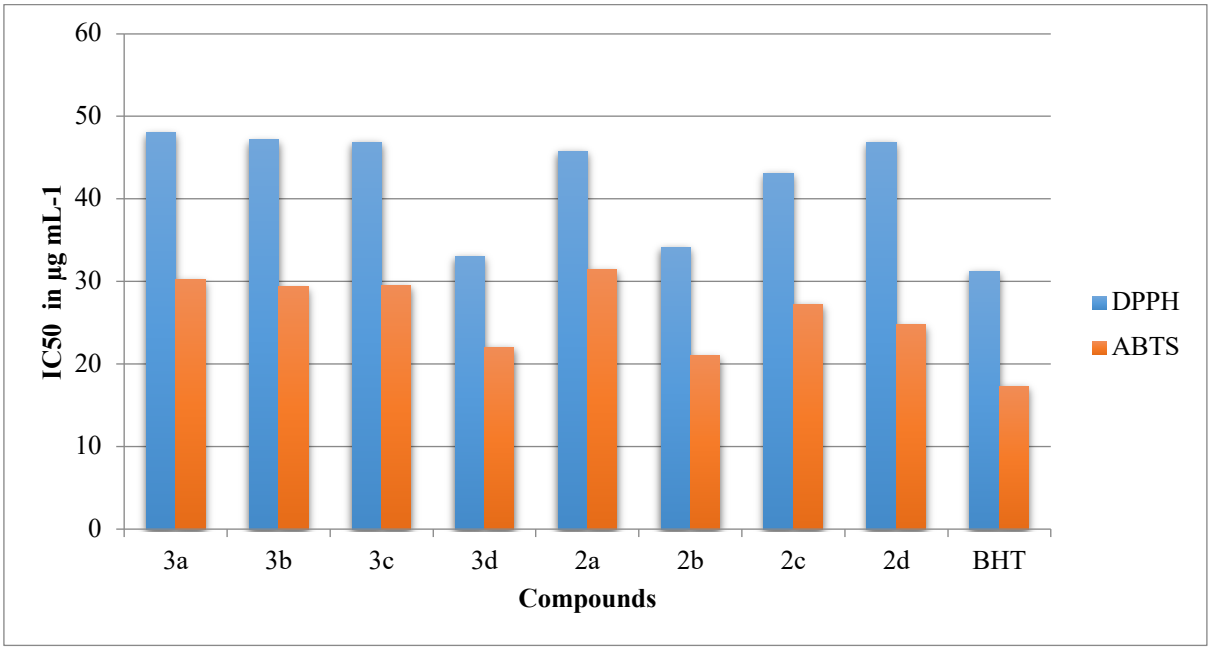

Figure 6. The antioxidative activity of compounds **2a-d** and **3a-d** synthesized was assessed by DPPH and ABTS techniques and expressed as IC_{50} in g mL^{-1}. And As a control, the BHT was used.

4. Conclusion

In summary, ruthenium(II)-NHC complexes **3a-d** have been easily prepared by the reaction of silver(I)-NHC complexes as a carbene transfer reagent with $[RuCl_2(p\text{-cymene})]_2$ in dichloromethane at room temperature in good yields. The molecular structures of the benzimidazolium salts (**2a-2d**) and the Ru(II)–N-heterocyclic carbene (NHC) complexes **3a-d** have been characterized by elemental analysis and ^{1}H- and ^{13}C-NMR spectra. The obtained benzimidazolium salts (**2a-2d**) and Ru(II)–N-heterocyclic carbene (NHC) complexes **3a-3d** were tested for their antibacterial activity. Synthesized compounds **3b** and 4d exhibited strong inhibitory activity against all tested indicator microorganisms. The lowest MICs values were obtained with the two complexes **3b** and **3d**. Against C. albicans, Enzymatic inhibitory investigation against acetylcholinesterase (AChE) and tyrosinase (TyrE), showed that the two complexes **3b** and **3d** are the most potent inhibitors against (AchE) with an IC50 of 2.52 and 5.06 µg mL-1 respectively, and against (TyrE) with an IC_{50} of 19.88 and 24.95 µg mL-1 respectively. Antioxidant activity of the synthesized N-Heterocyclic carbene (NHC) ligands (**2a-2d**) and their respective ruthenium(II) complexes (**3a-3d**) was assessed by two different techniques: the 2.2-diphenyl-1-picrylhydrazyl (DPPH) and the 2.2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals scavenging showed that complex **3d** has recorded an important antioxydant activity. This activity is very strong and quasi-similar to that of the standard BHT.

Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] B.Rosenberg, L.Vancamp, J.E.Trosko, V.H. Mansour, Platinum compounds: A new class of potent antitumour agents,*Nature.**222***,385–386 (1969).

[2] S. Medici, M. Peana, V.M. Nurchi, J.I. Lachowicz, G. Crisponi, M. A. Zoroddu, Noble metals in medicine: Latest advances, Coord *Chem Rev.* **284**,329–350 (2015).

[3] A.L. Noffke, A. Habtemariam, A.M. Pizarro, P.J.Sadler, Designing organometallic compounds for catalysis and therapy,*Chem Commun.***48,**5219–5246 (2012).

[4] W.Liu, R.Gust, Update onmetal N-heterocyclic carbine complexes as potential anti-tumor metallodrugs, *Coord Chem. Rev.***329**,191–213(2016).

[5] H.W. Wanzlick, H.J. Schönherr, Direct synthesis of amercurysalt-carbene complex, *Angew Chem Int Ed.* **7**,141–142(1968).

[6] K. Öfele,1,3-Dimethyl-4-imidazolinyliden-(2-)pentacarbonylchrom, *J Organomet Chem.* **12**,42–43(1968).

[7] A.J .Arduengo, R.L.Harlow, M. Kline ,A stable crystalline carbene, *J Am Che. Soc.* **113**,361–363(1991).

[8] W.A. Herrmann, N-Heterocycliccarbenes: A new concept inorganometallic catalysis, *Ange. Chem Int Ed.* **41**,1290–1309(2002).

[9] B Çetinkaya, E. Çetinkaya H. Küçükbay, R. Durmaz ,Antimicrobial activity of carbine complexes of rhodium(I) and ruthenium(II), *Arzneim.-Forsch./Drug Res.* **46**,821–823(1996).

[10] A .Melaiye, R.S .Simons, A. Milsted F.Pingitore, C. Westemiotis, C. A .Tessier, W.J. Youngs, Formation of water-soluble Pincer silver(I)-carbene complexes: A novel antimicrobial agent, *J Med Chem.* **47**,973–977(2004).

[11] P.J. Barnard, M.V. Baker, S.J. Berners-Price, D.A. Day, Mitochondrial permeability transition induced by dinuclear gold(I)-carbene complexes: Potential new antimitochondrial antitumour agents*, J Inorg Biochem.***9**,1642–1647(2004).

[12] M.E.Garner, W. Niu, X. Chen, I .Chiviriga, K.A. Abboud, W .Tan, A.S. Veige ,N-Heterocyclic carbine gold(I) and silver(I) complexes bearing functional groups forbio-conjugation, *Dalton Trans.* **44**,1914–1923(2015).

[13] C.V. Maftei, E .Fodor, P.G. Jones, M .Freytag, M .H .Franz, G .Kelter, H. H. Fiebig, M .Tamm, I. Neda ,N-Heterocyclic carbenes(NHC) with 1,2,4-oxadiazole-substituents related to natural products: Synthesis, structure and potential antitumor activity of some corresponding gold(I) and silver(I) complexes, *Eur J Med Chem.* **101**,431–441(2015).

[14] I. Briguglio, S .Piras, P. Corona, E .Gavini, M. Nieddu, G. Boatto, A. Carta, Benzotriazole: A noverviewonits versatile biological behavior, *Eur J Med Chem.* **97**,612–648(20]15).

[15] Z .He, S.F.Zhang, J.R. Xue, Y .Liang, X .Zhang, L.H. Jing, D.B. Qin, Verstile silver(I) and nickel(II) NHC complexes bearing benzotriazole-function: Synthesis, fluorescence and catalytic properties, *J Organomet Chem.* **808**,12–22(2016).

[16] M.Monticelli, S.Bellemin-Laponnaz, C.Tubaro, M. Ranca, Synthesis, structure and antitumoural activity of triazole functionalized NHC-metal complexes, *Eur J Inorg Chem.* **2017**,2488–2495(2017).

[17] G .Onar, M.O .Karataş, S .Balcıoğlu, T.T. Tok, C. Gürses, I. Kılıç-Cıkla, N .Özdemir, Ateş, B .Alıcı, Benzotriazole functionalized N-heterocyclic carbene-silver(I) complexes: Synthesis, cytotoxicity, antimicrobial, DNA binding and molecular docking studies, *Polyhedron* .**153**,31–40(2018).

[18] A .Bergamo, G. Sava, Ruthenium anticancer compounds : myths and realities of the emerging metal-based drugs, *Dalton Trans*.**40**,817-7823(2011).

[19]B.Tang , B.J.Han, D.Wan. *et al,* Synthesis, biological activities studies of ruthenium(II) polypyridyl complexes, *Transit Met Chem.* **42,**373–386(2017).

[20] G .Golbaghi, I Pitard, M. Lucas, M.M .Haghdoost, Y.L. de Los Santos, N. Doucet, S.A .Patten, T.J.Sanderson, A. Castonguay, Synthesis and biological assessment of a ruthenium(II) cyclopentadienyl complex in breast cancer cells and on the development of zebrafish embryos, *Eur J .Med .Chem*. **15**,188(2019).

[21] M. Małecka, A .Skoczyńska, D.M. Goodman, C.G. Hartinger, E. Budzisz ,Biological properties of ruthenium(II)/(III) complexes with flavonoids as ligands, *Coord Chem Rev*.**436**(2021).

[22] Z .Fudo, P.A. Ajibade, A.T. Odularu, Synthesis, Characterization, and Electrochemical Activities of Ruthenium(II) Bipyridyl-Dithiocarbamate Complexes, *Int. J .Photoenergy*.**10**, (2022).

[23] Y .Qui, Q. Peng ,Ruthenium sensitizers and their applications in dye-sensitized solar cells*, Int J Photoenergy.* **21** (2012).

[24] A.Anthonysamy, S.Balasubramanian, V.Shanmugaiah, N.Mathivanan ,Synthesis, characterization and electrochemistry of 4′-functionalized 2,2′:6′,2″-terpyridine ruthenium(II) complexes and their biological activity, *Dalton Trans*. **16**,2136–2143(2008).

[25] S .Moubeen, M.El-Shahat, A. Aziz, A.Attia, Synthesis, characterization and biological evaluation of novel octahedral Ru(III) complexes containing pentadentate Schiff base ligands, *Curr Chem Lett*. **10**,17-32(2021).

[26] N. Touj, I.S. Al Nasr, W.Koko, T. Khan, I. Özdemir, S. Yasar, L.Mansour, F.Alresheedi, N. Hamdi ,Anticancer, antimicrobial and antiparasitical activities of copper(I) complexes based on N-heterocyclic carbene(NHC) ligands bearing aryl substituents, *J Coord Chem.***73**,2889–2905(2020).

[27] I .Al Nasr, N .Touj, W. Koko, T.Khan, I Özdemir, S .Yasar , N. Hamdi, Biological Activities of NHC–Pd(II) Complexes Based on Benzimidazolylidene N-heterocyclic Carbene (NHC) Ligands Bearing Aryl Substituents, *Catalysts*.**10**,1190 (2020).

[28] L.Boubakri, A. Chakchouk-Mtiba, O.Naouali, L.Mellouli, L. Mansour, I. Özdemir, S.Yaser, M .Sauthier, N. Hamdi ,Ruthenium(II) complexes bearing benzimidazole-based N-heterocyclic carbene(NHC) ligands as potential antimicrobial, antioxidant ,enzymeinhibition, and antiproliferative agents, *J Coord Chem.***75**, 645–667(2022).

[29] K .Guven, E .Yucel, F. Cetintas, Antimicrobial Activities of Fruits of Crataegus. and Pyrus. *Species,Pharm Biol*. **44**,79(2006).

[30] I .Sellem, I. Kaaniche, A .Chakchouk-Mtibaa, L .Mellouli, Anti-oxidant, antimicrobial and anti-acetylcholinesterase activities of organic extracts from aerial parts of three Tunisian plants and correlation with polyphenols and flavonoids contents, *Bangladesh J Pharmacol*. 11, 531 (2016).

[31] G.L.Ellman, K.D.Courtney, V. Andres, R.M. Feather-Stone, *Biochem. Pharmacol*. **7**,88(1961).

[32] N.Rangkadilok, S.Sitthimonchai, L.Worasuttayangkurn, C.Mahidol, Ruchirawat, M.,J Satayavivad. *Food Chem. Toxicol.* ***45***,328(2007).

[33] A.J. Kirby, R.J. Schmidt,The antioxidant activity of Chinese herbs for eczema and of placebo herbs-I,*J. Ethnopharmacol.* **56**,103(1997).

[34] P.Re, R. Proteggente, N.Pannala, A. Yang, C.M.A .Rice-Evans. Free Radic, *Biol Med*. **26**, 336(1999).

1 [35] E .O.Karaca, I. Z. Dehimat, S.Yasar, N.Gürbüz, D .Tebbani, B.Çetinkaya, I. Ozdemir, An Efficient and Selective Nickel-Catalyzed Direct N-Alkylation of Anilines with Alcohols, *Inorganica Chimica Acta*. **520**,120294(2021).

[36] G.Onar, C.Gürses, M.O.Karataş, S.Balcıoğlu, N.Akbay, N. Özdemir, B.Ateş, B. Alıcı ,Palladium(II) and ruthenium(II) complexes of benzotriazole functionalized N-heterocycliccarbenes: Cytotoxicity, antimicrobial, and DNA interaction studies, *J Organomet Chem.***886**,48–56(2019).

[37] M.O.Karataş, B.Olgundeniz, S.Günal, İ.Özdemir, B.Alıcı, E. Çetinkaya,Synthesis, characterization and antimicrobial activities of novel silver(I) complexes with coumarin substituted N-heterocyclic carbene ligands, Bioorg Med Chem. 24,643-650(2016).

[38] I. Slimani, A .Chakchouk-Mtibaa, L. Mansour, L. Mellouli, I. Özdemir, N .Gürbüzd, N.Hamdi, Synthesis, characterization, biological determination and catalytic evaluation of ruthenium(ii) complexes bearing benzimidazole-basedNHC ligands intransfer hydrogenation catalysis, *New J Chem.****44***,5309–5323(2020).

[39] D. Balasubramanian, L .Harper, B.Shopsin, V.J .Torres, *Staphylococcus aureus* pathogenesis in diverse host environments, Pathog.1,75 (2017).

[40] J .Pizarro-Cerda, P .Cossart, Microbe Profile: Listeria monocytogenes: a paradigm among intracellular bacterial pathogens, *Microbiology*. *165*,719-721 (2019).

[41]N.M.Moore, M.L. Flaws, Epidemiology and Pathogenesis of *Pseudomonas aeruginosa* Infections, Am Soc Clin Lab Sci. **24** ,43-46(2011).
[42] J.A .Crump, S.P .Luby,Mintz ,The global burden of typhoid fever*, Bull. World Health Organ.82*,346–353(2004).
[43] G.Molero, R.Diez-Orejas, F.Navarro-Garcia, L.Monteoliva, J .Pla, C .Gil, M. Sánchez-Pérez, C. Nombela ,Candida albicans: Genetics, dimorphism and pathogenicity, *Int Microbiol***.1**,95–106(1998).
[44] C.N .Ciurea, I.B.Kosovski, A.D .Mare, F. Toma, I.A. Pintea-Simon, A.Man ,*Candida* and Candidiasis—Opportunism Versus Pathogenicity, A Review of the Virulence Traits. *Microorganisms*.**8**,857-874(2020).
[45] M.G. Lionetto, R .Caricato, A. Calisi, M.E.Giordano, T Schettino, Acetylcholinesterase as a Biomarker in Environmental and Occupational Medicine: New Insights and Future Perspectives, *BioMed Res* .(2013).
[46] W.A.T.Atta-ur-Rahman, S.A.Nawas, M.I.Choudhary, New cholinesterase inhibiting bisbenzylisoquinoline alkaloids from Cocculus pendulus, *Chem Pharm Bull*. **52**, 802-806(2004).
[47] W .Ahmad, B .Ahmad, M. Ahmad, Z .Iqbal, M .Nisar, M.Ahmad, In vitro inhibition of acetylcholinesterase, butyrylcholinesterase and lipoxygenase by crude extract of *Myricaria elegans Royle*, *J. Biol Sci*.**3**,1046-1049(2003).
[48] S. Zolghadri, A. Bahrami, M.T.H. Mahmud Tareq Hassan Khan, J.Munoz-Munoz, F .Garcia-Molina, F. Garcia-Canovas, A.A. Saboury, A comprehensive review on tyrosinase inhibitors, *J Enzyme Inhib Med Chem*. **34**,279–309(2019).
[49] V.M.Dembitsky, A.Kilimnik, Anti-melanoma agents derived from fungal species*, M. J. Pharma*. 1,1–16(2016).