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Evaluation of Ruthenium(II) *N*-Heterocyclic Carbene Complexes as Enzymatic Inhibitory Agents with Antioxidant, Antimicrobial, Antiparasitical and Antiproliferative Activity

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Abstract: A series of [RuCl$_2$(p-cymene)(NHC)] complexes were obtained by reacting [RuCl$_2$(p-cymene)]2 with in situ generated Ag-N-heterocyclic carbene (NHC) complexes. The structure of the obtained complexes was determined by the appropriate spectroscopy and elemental analysis. In addition, we evaluated the biological activities of these compounds as antienzymatic, antioxidant, antibacterial, anticancer, and antiparasitic agents. The results revealed that complexes **3b** and **3d** were the most potent inhibitors against AchE with IC$_{50}$ values of 2.52 and 5.06 μM mL^{-1}. Additionally, **3d** proved very good antimicrobial activity against all examined microorganisms with IZ (inhibition zone) over 25 mm and MIC (minimum inhibitory concentration) < 4 μM. Additionally, the ligand **2a** and its corresponding ruthenium (II) complex **3a** had good cytotoxic activity against both cancer cells HCT-116 and HepG-2, with IC$_{50}$ values of (7.76 and 11.76) and (4.12 and 9.21) μM mL^{-1}, respectively. Evaluation of the antiparasitic activity of these complexes against *Leishmania major* promastigotes and *Toxoplasma gondii* showed that ruthenium complexes were more potent than the free ligand, with an IC$_{50}$ values less than 1.5 μM mL^{-1}. However, **3d** was found the best one with SI (selectivity index) values greater than 5 so it seems to be the best candidate for antileishmanial drug discovery program, and much future research are recommended for mode of action and in vivo evaluation. In general, Ru-NHC complexes are the most effective against *L. major* promastigotes.

Keywords: N-Heterocyclic carbene; 5,6-dimethylbenzimidazolium salts; ruthenium; HCT-116; HepG-2; antimicrobial; antioxidant; enzyme inhibition; *Leishmania*; *Toxoplasma*

1. Introduction

Currently, the use of organometallic and inorganic compounds is very common in contemporary medication [1,2]. New organometallic complexes called N-Heterocyclic Carbene (NHC) complexes show promise drug formulation [3–7]. Çetinkaya et al. revealed the results of the first study on the biological functions of NHC complexes [8–11]. For this reason, several research teams have synthesized functionalized NHC complexes and investigated their biological activities [12–17]. In this regard, complexes of the ruthenium (II/III) type have been thoroughly studied as DNA binding, antibacterial, and anticancer agents [18–21]. In particular, ruthenium-type compounds have been investigated against various cancer cell lines as prospective substitutes for the well-known diamine-dichloroplatinum (II) in

the formulation of novel anticancer medicines (cisplatin) [22]. Under physiological circumstances, ruthenium can access the +2 and +3 oxidation states and can bind to cells' proteins, nucleic acids, sulfur, or oxygen-containing molecules [23–29]. Additionally, depending on the characteristics of their ligand, ruthenium complexes can optimize the kinetics of their interactions with cell components. As a result, ruthenium complexes ligands exchange rates are near to those of biological processes, making them ideally suited for use in a variety of therapeutic contexts. Therefore, ruthenium compounds may have greater cytostatic activity than platinum-based medications against a variety of cancer cells and may also be helpful against cisplatin-resistant cancer cells. In addition, a brand-new class of highly intriguing physiologically active compounds known as ruthenium complexes has developed. Among the most dangerous human pathogens, *Staphylococcus aureus* is an eminent human pathogen that can colonize the human host and cause severe life-threatening illnesses [30–37].

Our research group was also investigating metal complexes with anticancer activity against a various types of cancer cell lines [38–40]. We are currently investigating new functional NHC ligands that provide a favorable environment for the development and utilization of metal compounds. In this paper, we synthesized and characterized a new series of Ru(I) NHC complexes containing benzimidazoles. The structure of the new compound was characterized by various spectroscopic and analytical methods. Next, enzyme inhibition against AChE and TyrE, antioxidant against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and β-carotene bleaching test, various biological activities such as antimicrobial activity against Gram-positive, Gram-negative and *Candida albicans*, antiproliferative activity against colon cancer cell lines (HCT-116) and hepatocellular carcinoma cell lines (HepG-2), as well as antiparasitic activity against *Leishmania major* and *Toxoplasma gondii*, and cytotoxicity against Vero cells, were examined.

2. Results

2.1. Chemistry

2.1.1. Preparation of Benzimidazolium Salts **2a–d**

The synthesis of benzimidazolium salts (**2a–d**) as NHC precursors was carried out as previously described [41] (Scheme 1). By using ^{1}H NMR, ^{13}C NMR, FT-IR, and elemental analysis, the structures of the benzimidazolium salts **2a–d** were confirmed.

The ^{1}H NMR spectra of precursors **2a–d** show characteristic downfield shifts in the range δ 9.85–11.83 ppm for the NCHN protons due to the positive charge of the molecules [42].

The assigned structure was further supported by the benzimidazolium salt's ^{1}H NMR spectra. Sharp singlets representing the C(2)-H resonances were detected at 9.85, 10.34, 11.83, and 11.58 ppm for **2a–c**, respectively. Chemical shifts measured by ^{13}C NMR agreed with the suggested structure. At 141.3, 143.0, 143.1, and 152.8 ppm, the imino carbon is a characteristic singlet for the 1H decoupling mode of the benzimidazolium bromides **2a–d**. The aliphatic area of the ^{13}C NMR spectra displayed a sequence of peaks in the range of 20.76–35.18 ppm corresponding to resonances of the aliphatic carbon nucleus, whereas aromatic rings were seen in the range of 113.38–152.82 ppm. These numbers are fairly consistent with data that have already been published [43,44].
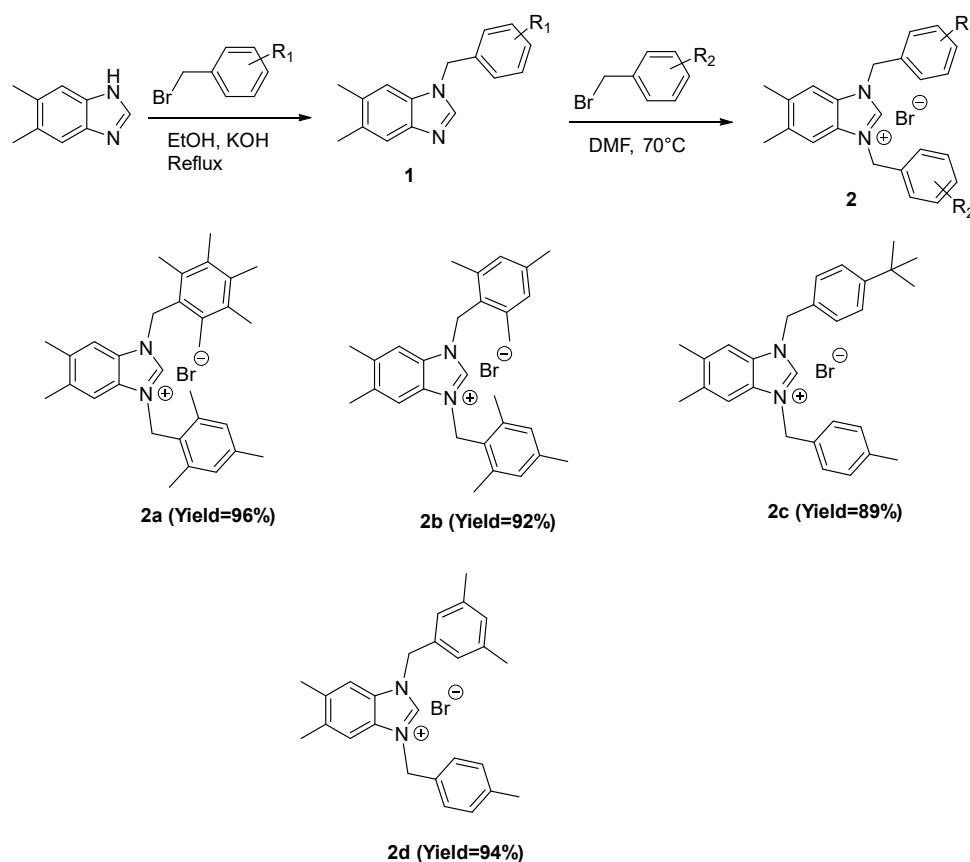
Scheme 1. General preparation of benzimidazolium salts (**2a–d**).

2.1.2. Preparation of Ruthenium-Carbene Complexes **3a–3d**

By transmetallating the corresponding silverNHC derivatives without isolation, the novel [RuCl₂(p-cymene)(NHC)] complexes (**3a–3d**) were prepared using a two-step procedure. By then adding [RuCl₂(p-cymene)]2 to the mixture, orange–brown complexes were obtained with high yields (80–90%). In contrast to nonpolar solvents, chloroform, dichloromethane, tetrahydrofuran, and ruthenium carbene complexes (**3a–3d**) are soluble in these solvents. Scheme 2 provides the synthesis and the structures of Ru(II)-NHCs complexes. Using spectroscopic methods such as ¹H NMR, ¹³C NMR, and IR as well as elemental studies, the structures of complexes **3a–3d** were determined.

The aromatic protons of complexes **3a–3d** appeared as multiplets between 5.57–5.88 and 7.13–7.69 ppm, the methyl protons appeared between 0.92–1.16 and 2.27–2.61 ppm as singlets. In all complexes (**3a–3d**), the -CH proton of the p-cymene group was seen as a heptet in the 2.64–2.86 ppm range. (NCH₂) exhibited a doublet resonance in the ¹H NMR spectra of (**3a–3d**) between 4.21 and 5.01 and 5.29 and 5.31 ppm. The carbene carbon in the ruthenium complexes **3a–3d** exhibits 13C chemical shifts at 189.2, 187.8, 188.9, and 189.0 ppm, respectively. The values obtained are comparable to those that have been published for other Ru-NHC complexes [45,46]. The ruthenium complexes **3a–3d** were also validated by elemental analysis results.
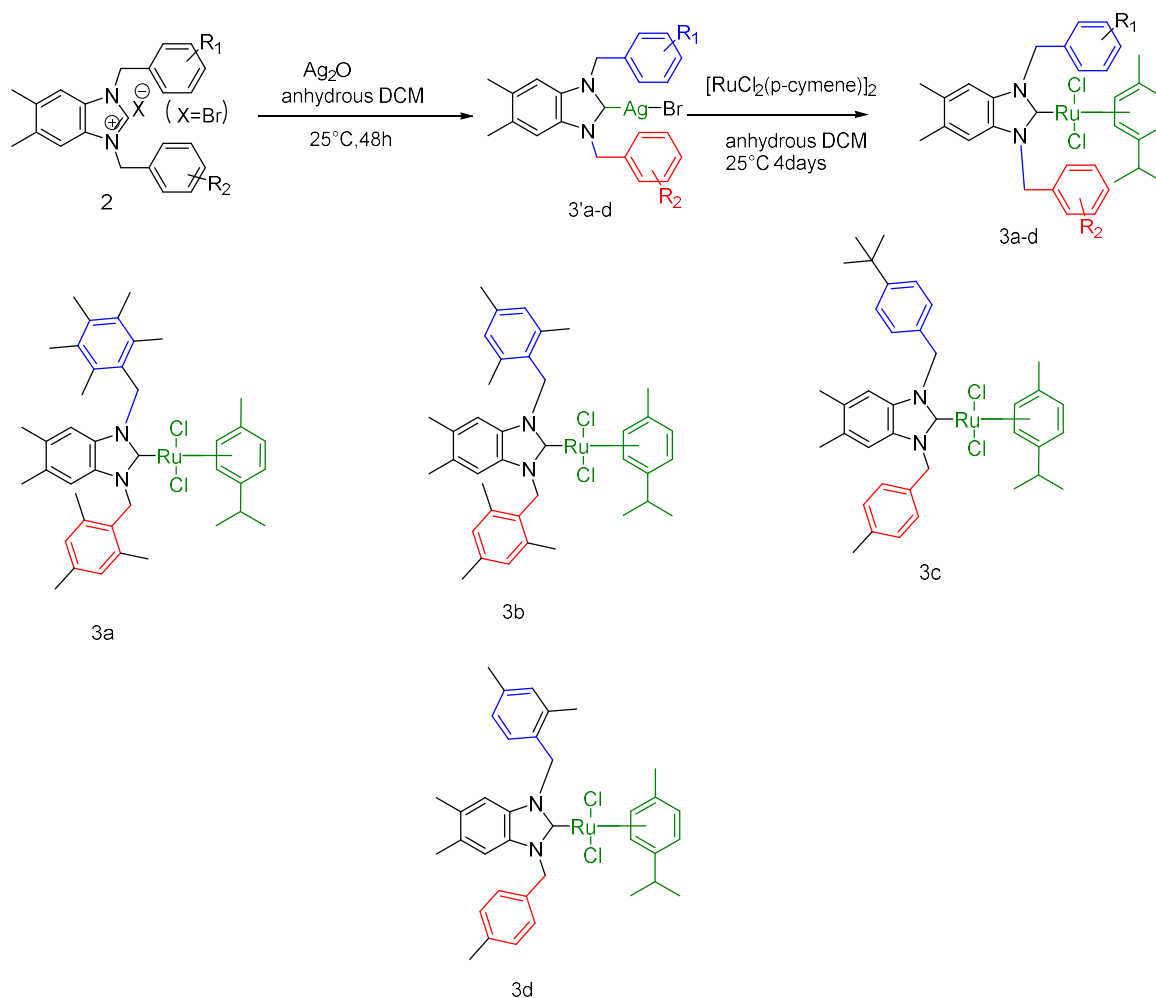
Scheme 2. General preparation of ruthenium N-heterocyclic carbene complexes **3a**–**3d**.

2.2. Biological Evaluation

2.2.1. Enzymatic Inhibitory, AChE and TyrE Inhibitory Activity

The results shown in Table 1 indicate that complexes **3b** and **3d** were the most potent inhibitors against AchE with IC_{50} values of 2.52 and 5.06 μM mL^{-1}.

Table 1. Anti-AChRi and anti-TyrEI inhibitory activity of the synthesized compounds IC_{50} in μM mL^{-1} of mean ± S.D.

| Compound	AChEI	TyrEI
2a	18.38 ± 2.7	45.05 ± 7.3
2b	13.41 ± 1.8	55.64 ± 8.2
2c	20.51 ± 3.2	48.01 ± 6.6
2d	22.27 ± 3.1	43.25 ± 6.8
3a	15.05 ± 2.2	50.75 ± 8.3
3b	2.52 ± 3.4	19.88 ± 2.4
3c	11.95 ± 1.6	38.17 ± 5.5
3d	5.06 ± 0.8	24.95 ± 3.7
Galanthamine	0.25 ± 0.04	-
Kojic acid	-	5.05 ± 0.8

2.2.2. Antioxidant Activity

A significant antioxidant activity, comparable to that of the conventional BHT, was found for compound **3d**. For the DPPH, ABTS, and -carotene tests, the IC_{50} values of this compound were 32.18, 18.17, and 92.25 μM mL^{-1}, respectively (Table 2). For the DPPH, ABTS, and -carotene tests, the standard BHT's IC_{50} values were 31.55, 17.41, and 89.55 μM mL^{-1}, respectively (Table 2).

Table 2. Antioxidant activity of the synthesized compounds assessed by DPPH, ABTS antiradical scavenging power and β-carotene bleaching test presented by their IC_{50} in μM mL^{-1} of mean ± S.D.

Compound	DPPH	ABTS	β-Carotene
2a	58.27 ± 7.4	39.12 ± 5.9	395.75 ± 51.6
2b	63.45 ± 10.1	38.14 ± 4.9	325.55 ± 48.7
2c	70.23 ± 12.0	51.27 ± 8.6	348.70 ± 52.1
2d	74.25 ± 11.6	55.21 ± 7.4	360.90 ± 40.8
3a	61.25 ± 10.3	41.78 ± 7.3	401.12 ± 66.5
3b	43.05 ± 5.7	32.05 ± 4.2	225.45 ± 31.5
3c	65.49 ± 11.2	45.04 ± 6.6	374.87 ± 55.7
3d	32.18 ± 5.4	18.17 ± 3.0	92.25 ± 11.9
BHT	31.55 ± 4.8	17.41 ± 2.9	89.55 ± 13.3

2.2.3. Antimicrobial Activity

Figure 1 displays the antibacterial activity of the corresponding ruthenium (II) complexes (**3a**–**3d**) and the synthetic NHC ligands (**2a**–**2d**). All of the examined indicator organisms were inhibited by complexes **3b** and **3d**. Inhibition zones caused by complex **3d** against *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *C. albicans* are 26, 28, 29, 27, 27, and 27 mm, respectively (Figure 1).
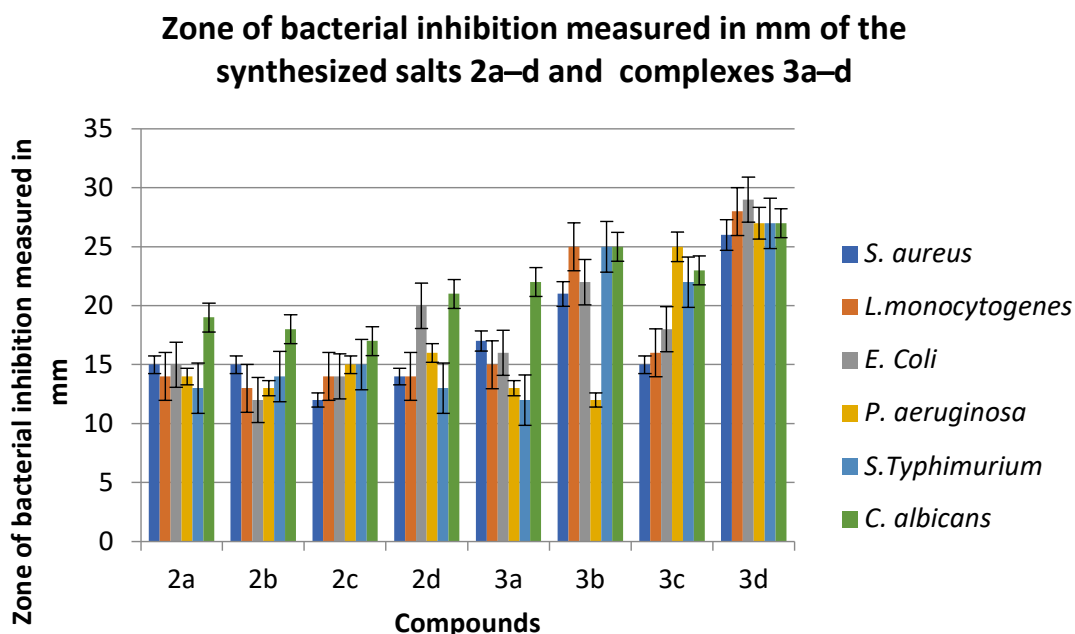
Figure 1. The antimicrobial activity of the synthesized compounds. The diameters of the inhibition zones were reported in mm.

The MIC ranges from 1.95 to 62.5 μM mL^{-1} for *S. aureus* and *S. typhimurium*, from 3.9 to 62.5 μM mL^{-1} for *L. monocytogenes*, and from 1.25 to 31.25 μM mL^{-1} for *C. albicans*. Complexes **3b** and **3d** gave the lowest MIC values. The MIC values for synthetic compound

3b against *L. monocytogenes*, *S. aureus*, *S. typhimurium*, and *C. albicans* were 15.6, 3.9, 3.9, and 1.25 μM mL^{-1}, respectively (Figure 2). In relation to complex **3d**, the MIC values against *L. monocytogenes*, *S. aureus*, *S. typhimurium*, and *C. albicans* were 3.9, 1.95, 1.95, and 1.25 μM mL^{-1}, respectively (Figure 2). Additionally, Figure 2 shows that complex **3d**'s MIC value against *L. monocytogenes* was the same as that of conventional ampicillin (3.9 g).
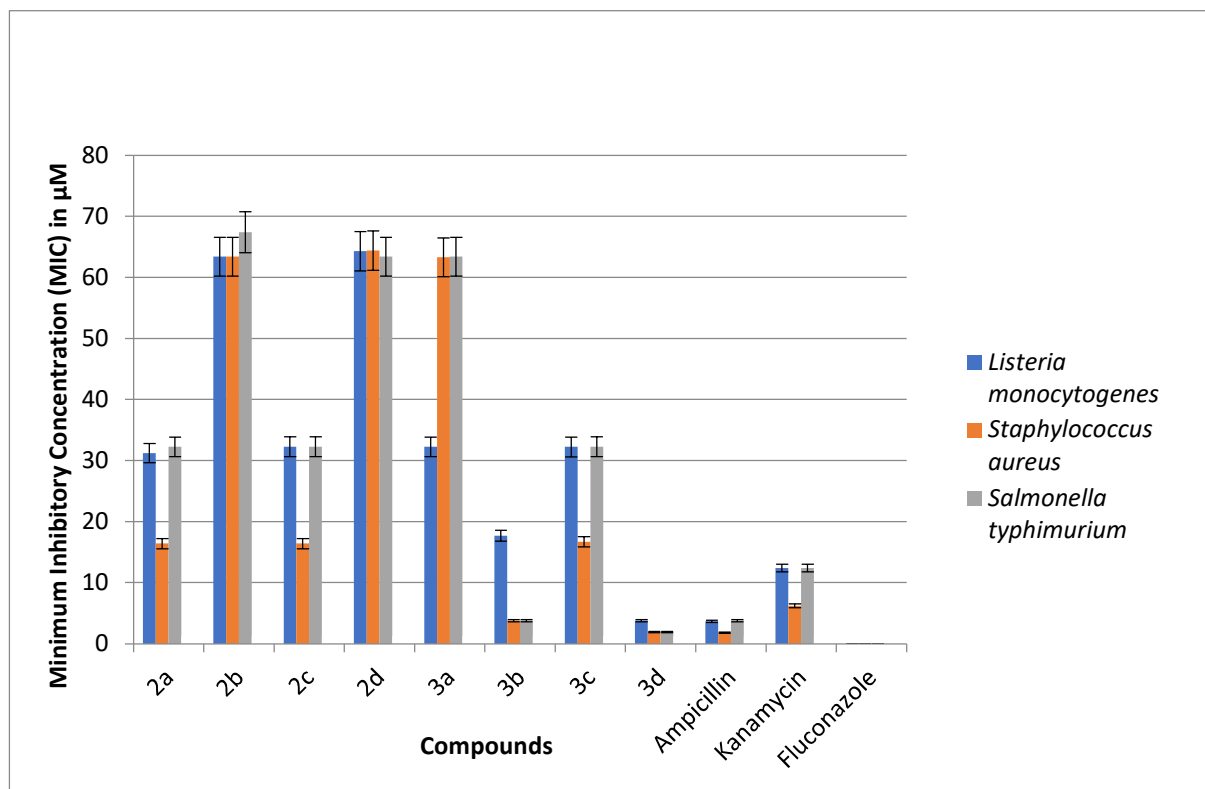
Figure 2. Minimum inhibitory concentration (MIC) of synthesized compounds against *L. monocytogenes*, *S. aureus* and *S. typhimurium*.

2.2.4. Antiproliferative Activity

Screening of the selected compounds against human colon carcinoma cancer cell lines and hepatocellular carcinoma cell lines revealed that the compound ruthenium(II) complex **3a** had IC$_{50}$ values (4.12 and 9.21 μM mL^{-1}) in both human cancer cell lines where the mentioned values were approximately equivalent to those of standard vinblastine drugs (3.83 and 6.05 μM mL^{-1}) in cytotoxic activity Table 3.

Table 3. IC$_{50}$ of the synthesized compounds on colon carcinoma cells (HCT-116) and hepatocellular carcinoma cell lines (HepG-2). IC$_{50}$ in μM mL^{-1} of mean ± S.D.

Compounds	c	
	HCT-116	**HepG-2**
2a	7.76 ± 1.1	11.75 ± 2.1
2b	13.56 ± 2.0	17.45 ± 2.7
2c	15.36 ± 2.6	20.65 ± 3.3
2d	16.04 ± 2.9	26.82 ± 3.6
3a	4.12 ± 0.6	9.21 ± 1.3
3b	19.45 ± 3.2	31.40 ± 4.8
3c	8.45 ± 1.2	12.73 ± 1.8
3d	8.45 ± 0.9	12.75 ± 1.7

2.2.5. Antiparasitical Activity

Antileishmanial Results

From Table 4, we can observe that all the compounds revealed antileishmanial activity against both the amastigote and promastigote stages. For amastigotes, all the compounds gave IC_{50} values less than 4.3 μM mL^{-1}, and only Compound **2b** showed IC_{50} values less than 1 μM mL^{-1} (0.3 μM mL^{-1}). All ruthenium(II) complexes (**3a–d**) gave IC_{50} values less than 1 μM mL^{-1} against *L. major* promastigotes. However, complexes **3c** and **3d** are the most active against *L. major* promastigotes, with SI values over five. There are strong similarities for the cytoxicity results of all compounds, with CC_{50} values in the range of 1.1 to 2.9 μM mL^{-1}. Therefore, only two compounds, **3a** and **3d**, can be recommended for future use as antileishmanial agents.

Table 4. Antileishmanial activity of selected compounds against *L. major* promastigotes and amastigotes in IC_{50} and CC_{50} of μM mL^{-1} of mean $\pm$ S.D.

Compound	CC_{50} Toxicity (Vero Cell)	Amastigote IC_{50}	Promastigotes IC_{50}	Amastigote SI	Promastigote SI
2a	1.2 ± 0.2	1.1 ± 0.1	3.2 ± 0.5	1.1	0.4
2b	1.1 ± 0.1	0.3 ± 0.04	2.2 ± 0.3	3.7	0.5
2c	2.9 ± 0.4	1.7 ± 0.2	5.8 ± 0.9	1.7	0.5
2d	1.7 ± 0.3	1.4 ± 0.2	1.9 ± 0.3	1.2	0.9
3a	1.2 ± 0.3	2.4 ± 0.4	0.45 ± 0.07	0.5	2.7
3b	1.3 ± 0.3	1.1 ± 0.2	0.74 ± 0.08	1.2	1.8
3c	1.9 ± 0.4	4.2 ± 0.8	0.37 ± 0.06	0.45	5.1
3d	1.8 ± 0.2	1.4 ± 0.3	0.32 ± 0.04	1.3	5.6

Antitoxoplasmal Results

Table 5 indicates that 4 compounds (ruthenium (II) complexes **3a–d**) showed antitoxoplasmic activity with IC_{50} values $\leq$ 1.5 μM mL^{-1}, which were 1.3, 1.4, 1.5 and 1.4 μM mL^{-1}, respectively. However, their SI was less than 1.5, which indicates their toxicity for Vero cells that can limit their future uses for drug formulation.

Table 5. Antitoxoplasmal activity of selected compounds against *T. gondii* in IC_{50} and CC_{50} of μM mL^{-1} of mean $\pm$ S.D.

Compound	CC_{50} Toxicity (Vero Cell)	*Toxoplasma* IC_{50}	SI *Toxoplasma*
2a	1.2 ± 0.2	8.4 ± 1.0	0.14
2b	1.1 ± 0.1	3.7 ± 0.5	0.3
2c	2.9 ± 0.4	2.1 ± 0.3	1.4
2d	1.7 ± 0.3	3.8 ± 0.5	0.4
3a	1.2 ± 0.3	1.3 ± 0.2	0.9
3b	1.3 ± 0.3	1.4 ± 0.2	0.9
3c	1.9 ± 0.4	1.5 ± 0.3	1.3
3d	1.8 ± 0.2	1.4 ± 0.1	1.3

3. Materials and Methods

All procedures were carried out under an inert atmosphere using standard Schlenk line techniques according to our previous work [38–40].

3.1. 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 couple hours. Then, the reaction mixture was filtred through filter paper, and the white solid was dried under vacuum, then crystallized with DCM-ether (1:3) for further purification.

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

m.p. 307.7 °C. Yield (96%). ν(CN) = 1440.99 cm^{-1}. ^{1}H NMR (CDCl$_3$, 400MHz) δ (ppm) 2.22(s, 30H, CH$_3$), 2.28(s, 6H, CH$_3$(a,b)), 5.8(s, 4H, CH$_{2(1',1'')}$), 7.11(s, 2H, Harom(4,7)),9.85(s, 1H, H$_2$). ^{13}CNMR(CDCl$_3$, 101MHz) δ (ppm) 17.01(CH$_3$), 16.92(C$_{(c,g,c',g')}$), 17.01 (C$_{(d,f,d',f')}$), 17.28(C(e,e')), 20.77(C$_{(a,b)}$), 48.10(C$_{(1';1'')}$), 113.41$_{(C4;7)}$, 125.58$_{(C8;9)}$, 130.42(C$_{4';5';6';4'';5'';6''}$), 133.50 (C$_{3';7';3'';7''}$), 133.74(C$_{5;6}$), 136.88(C$_{2',2''}$),141.35(C$_2$). Anal. Calcd for C$_{31}$H$_{39}$BrN$_2$:C, 71.66%; H, 7.57%; N, 5.39%%. Found: C, 71.17; H, 7.8; N, 5.4%.

5,6-dimethyl-1,3-bis(2,4,6-trimethylbenzyl)-1H-benzo[d]imidazol-3-ium bromide 2b

m.p. 210.6 °C. Yield (92%). ν(CN) = 1454.84 cm^{-1}. ^{1}H NMR (CDCl$_3$, 400MHz) δ (ppm) 2.15(s, 12H, CH$_3$(a,e,a',e')), 2.18(s, 12H, CH$_3$(b,d,b',d')), 2.20(s, 6H, CH$_3$(c,c')), 5.84(s, 4H, CH$_2$), 7.2(d, 2H, Harom(5,6)), 7.34(d, 2H, Harom(4,7)), 10.34(s, 1H, H$_2$). ^{13}C NMR(CDCl$_3$,100MHz) δ (ppm) 16.95(C$_{a,e,a',e'}$), 17.06(C$_{b,d,b',d'}$), 17.30(C$_{c,c'}$), 48.58(C$_{1';1''}$), 113.72(C$_{4;7}$), 125.36(C$_{5;6}$),126.88(C$_{8;9}$),131.85(C$_{5';5''}$),133.49(C$_{4';6';4'';6''}$), 133.82(C$_{3';7';3'';7''}$), 137.07(C$_{2',2''}$), 143(C$_2$). Anal. Calcd for C$_{29}$H$_{33}$BrN$_2$:C, 71.16%; H, 6.80%; N, 5.72%. Found: C, 71.3; H, 6.9; N, 5.8%.

5,6-dimethyl-1,3-((4-(tert-butyl)-4-methyl-benzyl)-1H-benzo[d]imidazol-3-ium bromide 2c

m.p. 296.5 °C. Yield (89%). ν(CN) = 1427.88 cm^{-1}. ^{1}H NMR (CDCl$_3$, 400MHz) δ (ppm) 1.2 (s, 18H, CH$_3$), 5.75(s, 4H, CH$_2$), 7.33(d, 4H, Harom(4',6',4'',6'')),7.39(d, 4H, Harom(3';7'3'',7'')), 7.46(d, 2H, Harom(5;6)), 7.55(d, 2H, Harom(4;7)), 11.83(s, 1H, H$_2$). ^{13}CNMR(CDCl$_3$,101 MHz) δ (ppm) 31.18(CH$_3$), 34.67(C$_{8';8''}$), 51.27(C$_{1';1''}$), 113.78(C$_{4;7}$), 126.34(C$_{4';6';4'';6''}$), 127.09(C$_{5,6}$), 128.17(C$_{3';7';3'';7''}$), 129.56(C$_{8;9}$),131.41(C$_{2',2''}$), 143(C$_2$),152.46 (C$_{5';5''}$). Anal. Calcd for C$_{28}$H$_{33}$BrN$_2$:C, 70.43%; H, 6.80%; N, 5.72%. Found: C, 70.2; H, 6.9; N, 5.8%.

5,6-dimethyl-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) ν(CN)(cm^{-1}): = 1565 (C=N); 1359 (C-N) cm^{-1} ; ^{1}H NMR (CDCl$_3$,300 MHz) δ (ppm):= 11.58 (s, 1H, H$_2$, NCHN); 7.44 (d, 2H, H$_{4'', 6''}$, arom. CH, 3 JHH = 7.43 Hz); 7.37 (s, 1H, H$_{5'}$, arom. CH); 7.35 (d, 2H, H$_{3'', 7''}$, arom. CH, 3 JHH = 7.34 Hz); 7.27 (s, 1H, H$_{3'}$, arom. CH); 7.01 (s, 2H, H$_{4,7}$, arom. CH); 6.94 (s, 1H, H$_{7'}$, arom. CH); 5.76 (s, 2H, H$_{1'}$,CH$_2$); 5.67 (s, 2H, H$_{1''}$,CH$_2$); 2.26 (s, 6H, H$_{d,e}$, 2 × CH$_3$); 1.25 (s, 9H, Ha,b,c, 3×CH$_3$). ^{13}C NMR (CDCl$_3$, 75MHz) (δ (ppm)):152. 81 (C$_2$, NCN); 142.37 (C$_{4', 6'}$, arom.Cq); 139.59 (C$_{2'}$, arom.Cq); 137.84 (C$_{5''}$, arom. CH); 133.11 (C$_{2''}$, arom.Cq); 131.35 (C$_{5, 6}$, arom.Cq); 130.45 (C$_{8, 9}$, arom.Cq); 128.53 (C$_{4'', 6''}$, arom. CH); 126.77 (C$_{3', 5', 7'}$, arom. CH); 126.23 (C$_{3'', 7''}$, arom. CH); 113.82 (C$_{4, 7}$, arom. CH); 51.80 (C$_{1'}$, CH$_2$); 51.43 (C$_{1''}$, CH$_2$); 35.17 (C$_{d, e}$, 2 × CH$_3$); 31.70 (Cc, CH$_3$); 21.75 (C$_{a, b}$, 2 × CH$_3$); Anal. Calcd for C$_{27}$H$_{31}$BrN$_2$:C, 69.97%; H, 6.74%; N, 6.04%. Found: C, 70.1; H, 6.8; N, 6.1%

Synthesis of ruthenium N-heterocyclic Carbene complexes (**3a–3d**): The synthesis was performed according to our previous work [38].

5,6-Dimethyl-[1,3-(2,3,4,5,6-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 (C-N).^{1}HNMR (300 MHz, CDCl$_3$, δ(ppm)): 7.13 (d, 2H, p-CH$_3$C$_6$H$_4$CH(CH$_3$)$_2$); 7.00 (d, 2H, p-CH$_3$C$_6$H$_4$CH(CH$_3$)$_2$); 6.92 (s, 1H, CH$_2$C$_6$H$_3$(CH$_3$)2-3,5); 6.84 (d, 4H, CH$_2$C$_6$H$_4$(CH$_3$)-4); 6.67 (s, 2H, CH$_2$ C$_6$H$_3$(CH$_3$)2-3,5); 6.51 (m, 2H, CH$_2$C$_6$H$_4$(CH$_3$)—4); 5.57 (m, 2H, C$_6$H$_2$(CH$_3$)2-5,6); 5.30 (s, 2H, H$_{1'}$, CH$_2$); 5.02 (s, 2H, H$_{1''}$, CH$_2$); 2.64 (p, 1H, H $_{7'''}$ pCH$_3$C$_6$H$_4$CH(CH$_3$)$_2$); 2.34 (s, 3H, He, CH$_3$); 2.30 (s, 6H, Hc, d, 2 × CH$_3$); 2.19 (s, 6H, Ha, b, 2 × CH$_3$); 1.82 (s, 3H, Hf, CH$_3$); 1.58 (s, 3H, Hi, CH$_3$); 1.15 (s, 6H, Hg, h, 2 × CH$_3$ (p-CH$_3$C$_6$H$_4$CH(CH$_3$)$_2$).^{13}C NMR (CDCl$_3$, 75 MHz) (δ (ppm)): 189.2 (C$_2$, NCN); 138.4; 138.2; 137.0; 135.0; 134.5; 134.3; 132.3; 129.6; 129.0; 125.8; 123.6; 111.9; 107.4; 96.6; 85.2; 52.4 (C$_{1'}$, CH$_2$); 52.2 (C$_{1''}$, CH$_2$); 30.6 (C$_{7'''}$, pCH$_3$C$_6$H$_4$CH(CH$_3$)$_2$); 21.6 (C$_{h, g}$, 2 × CH$_3$, p-CH$_3$C$_6$H$_4$CH(CH$_3$)$_2$); 21.2 (C$_{c, d}$, 2 × CH$_3$);

22.3 ($C_{e, f}$, 2 × CH_3); 18.1 ($C_{a, b, i}$, 3 × CH_3). Anal. Calcd for $C_{41}H_{53}RuN_2Cl_2$:C, 66.02%; H, 7.16%; N, 3.76%. Found: C, 66.1; H, 7.3; N, 3.8%.

5,6-Dimethyl-[1,3-(2,3,4,5,6-pentamethyl-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 (C-N).^{1}HNMR(300 MHz, CDCl$_3$, δ(ppm)): 7.69 (d, 2H, p-$CH_3C_6H_4CH(CH_3)_2$); 7.54 (d, 2H, p-$CH_3C_6H_4CH(CH_3)_2$); 7.26 (s, 1H, $CH_2C_6H_3(CH_3)_2$-3,5); 6.89 (s, 2H, $CH_2C_6H_3(CH_3)_2$); 6.63 (s, 2H, $C_6H_2(CH_3)_2$); 5.88 (s, 1H, $CH_2C_6H(CH_3)_4$); 4.21 (s, 4H, $H_{1',1''}$, 2 × CH_2); 2.86 (p, 1H, $H_{7'''}$, p-$CH_3C_6H_4CH(CH_3)_2$); 2.27 (s, 18H, $H_{a, b, c, d}$, f, g, 6 × CH_3); 2.20 (s, 3H, Hi, CH_3); 1.95 (s, 3H, H_l, CH_3); 1.32 (s, 6H, He,h, 2 × CH_3); 0.92 (s, 6H, Hj, k, 2 × CH_3 (p-$CH_3C_6H_4CH(CH_3)_2$). ^{13}C NMR (CDCl$_3$, 75 MHz) (δ (ppm)): 187.8 (C_2, NCN); 138.4; 135.4; 135.2; 134.5; 133.5; 133.2; 132.3; 131.6; 131.3; 130.0; 128.9; 124.6; 123.4; 107.1; 96.2; 54.0 (C1′, CH_2); 51.8 ($C_{1''}$, CH_2); 31.0 ($C_{7'''}$, p-$CH_3C_6H_4CH(CH_3)_2$); 21.5 ($C_{j, k}$, 2 × CH_3, p $CH_3C_6H_4CH(CH_3)_2$); 20.8 ($C_{c, d}$, 2 × CH_3); 20.2 (Ci, CH_3); 18.4 ($C_{f, g}$, 2 × CH_3); 16.3 ($C_{a, b}$, 2 × CH_3); 15.4 ($C_{e, h, l}$, 3 × CH_3). Anal. Calcd for $C_{41}H_{53}RuN_2Cl_2$:C, 66.02%; H, 7.16%; N, 3.76%. Found: C, 66.1; H, 7.3; N, 3.8%.

5,6-Dimethyl-[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)): 7.36 (d, 4H, $CH_2C_6H_4C(CH_3)$3-4); 7.03 (d, 4H, $CH_2C_6H_4C(CH_3)$3-4); 6.82 (s, 2H, $C_6H_2(CH_3)_2$-5,6); 6.55 (d, 2H, p $CH_3C_6H_4CH(CH_3)_2$); 5.62 (d, 2H, p-$CH_3C_6H_4CH(CH_3)_2$); 5.31 (s, 2H, $H_{1'}$, CH_2); 5.02 (s, 2H, $H_{1''}$, CH_2); 2.61(s, 6H, $H_{d, g}$, 2 × CH_3); 2.19(s, 6H, $H_{a, b}$, 2 × CH_3); 1.81 (s, 3H, Hi, CH_3); 1.66 (s, 1H, $H_{7'''}$, p-$CH_3C_6H_4CH(CH_3)_2$); 1.31 (s, 15H, $H_{c, e, f, h, l}$, 5 × CH_3); 1.12 (d, 6H, $H_{j, k}$, 2 × CH_3 (p$CH_3C_6H_4CH(CH_3)_2$). ^{13}CNMR (CDCl$_3$, 75 MHz) (δ (ppm)): 188.9 (C_2, NCN); 150.4; 134.9; 134.4; 132.3; 125.8; 125.5; 111.9; 106.8; 97.3; 85.5; 52.3 ($C_{1',1''}$, 2 × CH_2); 41.1 (C7′′′, p-$CH_3C_6H_4CH(CH_3)_2$); 34.6 (Cd, g, 2 × CH_3, $CH_2C_6H_4C(CH_3)_3$); 31.4 ($C_{c, e, f,h}$, 4 × CH_3); 30.5 (Ci, CH_3); 22.6 ($C_{a, b}$, 2 × CH_3); 20.3 ($C_{j, k}$, 2 × CH_3). DART-TOF-MS: *m/z* = 453, 426, 291. Anal. Calcd for $C_{38}H_{46}RuN_2Cl_2$:C, 64.95%; H, 6.60%; N, 3.99%. Found: C, 65.1; H, 6.7; N, 4.1%.

5,6-Dimethyl-[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)): 14 (d, 4H, $CH_2C_6H_4(CH_3)$-4); 7.00 (d, 4H, $CH_2C_6H_4(CH_3)$); 6.79 (s, 2H, $C_6H_2(CH_3)_2$); 6.46 (d, 2H, p-$CH_3C_6H_4CH(CH_3)_2$); 5.68 (d, 2H, p-$CH_3C_6H_4CH(CH_3)_2$); 5.29 (s, 2H, $H_{1'}$, CH_2); 5.03 (s, 2H, $H_{1''}$, CH_2); 2.68 (p, 1H, $H_{7'''}$, p-$CH_3C_6H_4CH(CH_3)_2$); 2.34 (s, 6H, $H_{c, d}$, 2 × CH3); 2.18 (s, 6H, Ha, b, 2 × CH_3); 1.84 (s, 3H, He, CH_3); 1.16 (d, 6H, $H_{f, g}$, 2 × CH_3 (p-$CH_3C_6H_4CH(CH_3)_2$). ^{13}CNMR (CDCl$_3$, 75 MHz) (δ (ppm)): 189.0 (C_2, NCN); 137.0; 134.9; 134.4; 132.3; 129.6; 125.9; 111.9; 107.6; 97.0; 85.30; 52.5 ($C_{1',1''}$, 2 × CH_2); 30.6 ($C_{7'''}$, p-$CH_3C_6H_4CH(CH_3)_2$); 22.6 ($C_{f, g}$, 2 × CH_3); 21.2 ($C_{c, d}$, 2 × CH_3); 20.3 (Ce, CH_3); 18.3 ($C_{a, b}$, 2 × CH_3); 14.2 (CH, CH_3).

3.2. Biological Activities

3.2.1. Enzymatic Inhibitory Assay

Acetylcholinesterase Inhibitory (AChEI)

According to the Ellman et al. published spectrophotometric method of electric eel AChE [47], AChEI activity was measured. Acetylthiocholine iodide (ATCI) was used as the reaction's substrate, and the antiacetylcholinesterase activity was measured using 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB).

Antityrosinase Activity

According to Rangkadilok et al. [48], the TyrE inhibitory activity was measured spectrophotometrically using L-tyrosine as the substrate in a 96-well microplate.

3.2.2. Antioxidant Activity

Three methods were used for the assessing antioxidant properties of the selected compounds, which are 2.2-diphenyl-1-picrylhydrazyl (DPPH), 2.2'-azino-bis (3-ethylbenzo thiazoline-6-sulphonic acid) (ABTS) radicals scavenging, and β-carotene linoleic acid bleaching assay. Antioxidant activity was expressed as IC_{50} (the concentration that causes 50% of inhbition effect). The control compound was butylated hydroxytoluene (BHT), which is a potent antioxidant.

DPPH Radical Scavenging Activity

Briefly, various amounts of produced compounds were diluted with ultrapure water after being dissolved in dimethylsulfoxide (DMSO)/water (1/9; *v/v*) (1, 0.5, 0.250, 0.125, 0.0625, 0.03125 mg mL^{-1}). The samples were then combined with 500 mL of a 4% (*w/v*) solution of the DPPH radical in ethanol. The combination was incubated for 30 min. at room temperature and in the dark [49]. Spectrophotometric analysis was used to calculate the scavenging capacity by comparing the decrease in absorbance at 517 nm to a blank.

ABTS Assay

ABTS radical scavenging activity was conducted by referring to the method of Re et al. [50].

β-Carotene Bleaching Assay

β-Carotene bleaching test was conducted following the method described by Pratt's [51].

All the assays used for antioxidant determination (DPPH, ABTS, and β-carotene bleaching assay) were performed simultaneously three times in the same conditions. The results obtained in μM mL^{-1} average of the three experiments.

3.2.3. Antimicrobial Activity

Microorganisms, media and growth conditions, agar well diffusion method for inhibition zone determination (IZ) and minimum inhibitory concentration (MIC) were performed according to the literature work [52,53].

The synthesized compounds were examined in vitro for their antimicrobial activity against Six standard microorganisms of ATCC, two Gram positive bacteria *S. aureus* and *Listeria monocytogenes*, three Gram negative bacteria *Esherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium* and the fungus *C. albicans.* Bacteria were cultured in Luria-Bertani (LB) medium, while Sabouraud agar was used for culturing *C. albicans* and the assay conducted according to our previous techniques [53]. The results were the average of 3 readings.

3.2.4. In Vitro Anticancer Proliferation Studies

The selected compound was investigated for its cytotoxic properties against HCT-116 and HepG-2 (cancer cell lines of ATCC, Rockville, MD, USA). Vinblastine was applied as reference drug. The assay was conducted according to the methods described by Mossman [54] and our recently published data [55].

The results presented IC_{50} (The concentration that causes 50% inhibitory of cell viability) of μM mL^{-1} from the average of 3 reading.

3.2.5. Antiparasitical Assessment
Leishmania Major Cell Isolation, Culture Conditions, and Assays

This assay was carried out according to the methods mentioned in our previously published article [56]. *L. major* promastigotes were isolated locally from an indoor patient in 2016, liquid nitrogen was used for the preservation of the parasites, and BALB/c mice were used for the maintenance of the parasites and production of *L. major* amastigotes. Phenol red-free RPMI 1640 medium (Invitrogen, USA) with 10% FBS was used for the culture and in vitro evaluation, while amphotericin B (AmB) was used as reference drug. The result

was expressed in IC_{50} values (the concentration that causes 50% inhibition of the viable parasites) of three independent readings, followed by the selectivity index (SI) calculation by dividing CC_{50} (toxic concentration that causes 50% inhibition of cell growth) over IC_{50} of the same compound [56].

Toxoplasma Gondii Cell Line, Culture Conditions, and Assay

This assay was carried out according to the methods mentioned in our previously published article [50]. Vero cells line (ATCC® CCL81™, USA) were used for the serial passage and cultivation of *T. gondii* tachyzoites RH strain, complete RPMI 1640 medium with heat-inactivated 10% FBS was used for the culture and in vitro evaluation, while atovaquone (ATO) was used as reference drug. The results were expressed in IC_{50} of three independent readings, followed by the selectivity index (SI) calculation by dividing CC_{50} over IC_{50} of the same compound [56].

3.2.6. In Vitro Cytotoxicity Assay

MTT colorimetric technique was carried out for cytotoxicity evaluation according to the methods mentioned in our previously published article [57]. An amount of 96 well plates with complete were used for the culture of the cells. FLUOstar OPTIMA spectrophotometer was applied for colorimetric analysis and in vitro evaluation. Cytotoxic effects were expressed by CC_{50} values (concentration that caused a 50% reduction in viable cells), from three independent experiments [56].

4. Conclusions

In summary, ruthenium(II)-NHC complexes **3a**–**3d** have been easily prepared by the reaction of silver(I)- NHC complexes as a carbene transfer reagent with [RuCl2(p-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**–**3d** were characterized by elemental analysis and 1H- and ^{13}C-NMR spectra.

The results of the enzymatic inhibitory study against AChE and TyrE revealed that complexes **3b** and **3d** are the most effective inhibitors against AchE, with respective IC_{50} values of 2.52 and 5.06 μM mL^{-1} and 19.88 and 24.95 μM mL^{-1}. These results confirm that NHC metallic complexes have potent antibacterial properties [58]. Important antioxidant activity was observed for Complex **3**. The synthesized NHC ligands (**2a**–**2d**) and their corresponding ruthenium(II) complexes (**3a**–**3d**) were screened against HCT-116 and HepG-2, and the results revealed that ruthenium(II) complex **3a** exhibited cytotoxic activity approximately equivalent to that of standard vinblastine, so we can suggest ruthenium(II) complex **3a** can be used in the formulation of drugs that stimulate cancer treatment against human colon carcinoma cancer and liver hepatocellular carcinoma cancer after further pharmacological and clinical trials investigations. Regarding the last experiment of studying the ruthenium (II) complex as an antiparasitical agent against *L. major* and *T. gondii*, compounds **3c** and **3d** were found to have extremely potent antileishmania effects, with a SI over five, while all tested compounds had less antitoxoplasmic activity. These findings were similar to our previous investigation with NHC palladium complexes as well as the similar ruthenium complexes [53,57]. We propose that **3d** can be used as a drug candidate for many antimicrobial, anticancer, and antiparasite bioactivities, and further investigation for mode of action detection and in vivo evaluation is highly recommended.

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References

1. Rosenberg, B.; Vancamp, L.; Trosko, J.E.; Mansour, V.H. Platinum compounds: A new class of potent antitumour agents. *Nature* **1969**, *222*, 385–386. [CrossRef] [PubMed]
2. Medici, S.; Peana, M.; Nurchi, V.M.; Lachowicz, J.I.; Crisponi, G.; Zoroddu, M.A. Noble metals in medicine: Latest advances, Coord. *Chem. Rev.* **2015**, *284*, 329–350. [CrossRef]
3. Noffke, A.L.; Habtemariam, A.; Pizarro, A.M.; Sadler, P.J. Designing organometallic compounds for catalysis and therapy. *Chem. Commun.* **2012**, *48*, 5219–5246. [CrossRef] [PubMed]
4. Liu, W.; Gust, R. Update on metal N-heterocyclic carbene complexes as potential anti-tumor metallodrugs. *Coord. Chem. Rev.* **2016**, *329*, 191–213. [CrossRef]
5. Wanzlick, H.W.; Schönherr, H.J. Direct synthesis of a mercury salt-carbene complex. *Angew. Chem. Int. Ed.* **1968**, *7*, 141–142. [CrossRef]
6. Öfele, K. 1,3-Dimethyl-4-imidazolinyliden-(2-)pentacarbonylchrom. *J. Organomet. Chem.* **1968**, *12*, 42–43. [CrossRef]
7. Arduengo, A.J.; Harlow, R.L.; Kline, M. A stable crystalline carbene. *J. Am. Chem. Soc.* **1991**, *113*, 361–363. [CrossRef]
8. Herrmann, W.A. N-Heterocyclic carbenes: A new concept in organometallic catalysis. *Angew. Chem. Int. Ed.* **2002**, *41*, 1290–1309. [CrossRef]
9. Çetinkaya, B.; Çetinkaya, E.; Küçükbay, H.; Durmaz, R. Antimicrobial activity of carbene complexes of rhodium(I) and ruthenium(II). *Arzneim.Forsch. Drug Res.* **1996**, *46*, 821–823.
10. Melaiye, A.; Simons, R.S.; Milsted, A.; Pingitore, F.; Westemiotis, C.; Tessier, C.A.; Youngs, W.J. Formation of water-soluble Pincer silver(I)-carbene complexes: A novel antimicrobial agent. *J. Med. Chem.* **2004**, *47*, 973–977. [CrossRef]
11. Barnard, P.J.; Baker, M.V.; Berners-Price, S.J.; Day, D.A. Mitochondrial permeability transition induced by dinuclear gold(I)-carbene complexes: Potential new antimitochondrial antitumour agents. *J. Inorg. Biochem.* **2004**, *98*, 1642–1647. [CrossRef] [PubMed]
12. Garner, M.E.; Niu, W.; Chen, X.; Chiviriga, I.; Abboud, K.A.; Tan, W.; Veige, A.S. N-Heterocyclic carbene gold(I) and silver(I) complexes bearing functional groups for bio-conjugation. *Dalton Trans.* **2015**, *44*, 1914–1923. [CrossRef] [PubMed]
13. Maftei, C.V.; Fodor, E.; Jones, P.G.; Freytag, M.; Franz, M.H.; Kelter, G.; Fiebig, H.H.; Tamm, M.; Neda, I. 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.* **2015**, *101*, 431–441. [CrossRef]
14. Briguglio, I.; Piras, S.; Corona, P.; Gavini, E.; Nieddu, M.; Boatto, G.; Carta, A. Benzotriazole: An overview on its versatile biological behaviour. *Eur. J. Med. Chem.* **2015**, *97*, 612–648. [CrossRef]
15. He, Z.; Zhang, S.F.; Xue, J.R.; Liang, Y.; Zhang, X.; Jing, L.H.; Qin, D.B. Verstile silver(I) and nickel(II) NHC complexes bearing benzotriazole-function: Synthesis, fluorescence and catalytic properties. *J. Organomet. Chem.* **2016**, *808*, 12–22. [CrossRef]
16. Monticelli, M.; Bellemin-Laponnaz, S.; Tubaro, C.; Rancan, M. Synthesis, structure and antitumoural activity of triazole functionalized NHC-metal complexes. *Eur. J. Inorg. Chem.* **2017**, *2017*, 2488–2495. [CrossRef]
17. Onar, G.; Karataş, M.O.; Balcıoğlu, S.; Tok, T.T.; Gürses, C.; Kılıç-Cıkla, I.; Özdemir, N.; Ateş, B.; Alıcı, B. Benzotriazole functionalized N-heterocyclic carbene-silver(I) complexes: Synthesis, cytotoxicity, antimicrobial, DNA binding and molecular docking studies. *Polyhedron* **2018**, *153*, 31–40. [CrossRef]
18. Oehninger, L.; Stefanopovlou, M.; Alborzinia, H.; Schur, J.; Ludewig, S.; Namikawa, K.; Munoz-Castro, A.; Köster, R.W.; Baumann, K.; Wolfl, S.; et al. Evaluation of arene ruthenium(II) N-heterocyclic carbene complexes as organometallics interacting with thiol and selenol containing biomolecules. *Dalton Trans.* **2013**, *42*, 1657–1666. [CrossRef]
19. Ray, S.; Mohan, R.; Singh, J.K.; Samantaray, M.K.; Shaikh, M.M.; Panda, D.; Ghosh, P. Anticancer and antimicrobial metallopharmaceutical agents based on palladium, gold, and silver N-heterocyclic carbene complexes. *J. Am. Chem. Soc.* **2007**, *129*, 15042–15053. [CrossRef]

20. Streciwilk, W.; Terenzi, A.; Cheng, X.; Hager, L.; Dabiri, Y.; Prochnow, P.; Bandow, J.E.; Wölfl, S.; Keppler, B.K.; Ott, I. Fluorescent organometallic rhodium(I) and ruthenium(II) metallodrugs with 4-ethylthio-1,8-naphthalimide ligands: Antiproliferative effects,cellular uptake and DNA-interaction. *Eur. J. Med. Chem.* **2018**, *156*, 148–161. [CrossRef]

21. Teyssot, M.L.; Jarrousse, A.S.; Manin, M.; Chevry, A.; Roche, S.; Norre, F.; Beaudoin, C.; Morel, L.; Boyer, D.; Mahiou, R.; et al. Metal-NHC complexes: A survey of anti-cancer properties. *Dalton Trans.* **2009**, *2009*, 6894–6902. [CrossRef] [PubMed]

22. Rilak, A.; Bratsos, I.; Zangrando, E.; Kljun, J.; Turel, I.; Bugarčic, Ž.D.; Alessio, E. New Water-Soluble Ruthenium(II) Terpyridine Complexes for Anticancer Activity: Synthesis, Characterization, Activation Kinetics, and Interaction with Guanine Derivatives. *Inorg. Chem.* **2014**, *53*, 6113. [CrossRef] [PubMed]

23. Vuradi, R.K.; Nambigari, N.; Pendyala, P.; Gopu, S.; Kotha, L.R.; Deepika, G.; Rani, V.M.; Sirasani, S. Study of Anti-Apoptotic mechanism of Ruthenium (II)Polypyridyl Complexes via RT-PCR and DNA binding. *Appl. Organometal. Chem.* **2020**, *34*, e5332. [CrossRef]

24. Ezhilarasu, T.; Balasubramanian, S. Synthesis, Characterization, Photophysical and Electrochemical Studies of Ruthenium(II) Complexes with 4′-Substituted Terpyridine Ligands and Their Biological Applications. *ChemistrySelect* **2018**, *3*, 12039. [CrossRef]

25. Jakupec, M.A.; Galanski, M.; Arion, V.B.; Hartinger, C.G.; Keppler, B.K. Antitumour metal compounds: More than theme and variations. *Dalton Trans.* **2008**, *2*, 183. [CrossRef]

26. Dabiri, Y.; Schmid, A.; Theobald, J.; Blagojevic, B.; Streciwilk, W.; Ott, I.; Wölfl, S.; Cheng, X. A Ruthenium(II) N-Heterocyclic Carbene (NHC) Complex with Naphthalimide Ligand Triggers Apoptosis in Colorectal Cancer Cells via Activating the ROS-p38 MAPK Pathway. *Int. J. Mol. Sci.* **2018**, *19*, 3964. [CrossRef] [PubMed]

27. Keene, F.R.; Smith, J.A.; Collins, J.G. Metal complexes as structure-selective binding agents for nucleic acids. *Coord. Chem. Rev.* **2009**, *253*, 2021. [CrossRef]

28. Lam, N.Y.S.; Truong, D.; Burmeister, H.; Babak, M.V.; Holtkamp, H.U.; Movassaghi, S.; Ayine-Tora, D.M.; Zafar, A.; Kubanik, M.; Oehninger, L.; et al. From Catalysis to Cancer: Toward Structure-Activity Relationships for Benzimidazol-2-ylidene-Derived N-Heterocyclic-Carbene Complexes as Anticancer Agents. *Inorg. Chem.* **2018**, *57*, 14427–14434. [CrossRef]

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

30. Gangadevi, V.; Muthumary, J. Preliminary studies on cytotoxic effect of fungal taxol on cancer cell lines. *Afr. J. Biotechnol.* **2007**, *6*, 1382–1386.

31. Movassaghi, S.; Singh, S.; Mansur, A.; Tong, K.K.H.; Hanif, M.; Holtkamp, H.U.; Söhnel, T.; Jamieson, S.M.F.; Hartinger, C.G. (Pyridin-2-yl)-NHC Organoruthenium Complexes: Antiproliferative Properties and Reactivity toward Biomolecules. *Organometallics* **2018**, *37*, 1575–1584. [CrossRef]

32. Dembitsky, V.M.; Kilimnik, A. Anti-melanoma agents derived from fungal species. *Mathews J. Pharm. Sci.* **2016**, *1*, 1–16.

33. Yilmaz, A.; Price, R.W.; Gisslen, M. Antiretroviral drug treatment of CNS HIV-1 infection. *J. Antimicrob. Chemother.* **2012**, *67*, 299–311. [CrossRef]

34. Ciurea, C.N.; Kosovski, I.B.; Mare, A.D.; Toma, F.; Pintea-Simon, I.A.; Man, A. Candida and Candidiasis—Opportunism Versus Pathogenicity: A Review of the Virulence Traits. *Microorganisms* **2020**, *8*, 857–874. [CrossRef] [PubMed]

35. Lionetto, M.G.; Caricato, R.; Calisi, A.; Giordano, M.E.; Schettino, T. Acetylcholinesterase as biomarkers in environmental and occupational medicine: New insights and future perspectives. *BioMed. Res. Inter.* **2013**, *2013*, 1–8. [CrossRef]

36. Yang, Y.; Guo, L.; Tian, Z.; Liu, X.; Gong, Y.; Zheng, H.; Ge, X.; Liu, Z. Imine-N-Heterocyclic Carbenes as Versatile Ligands in Ruthenium(II) p-Cymene Anticancer Complexes: A Structure-Activity Relationship Study. *Chem. Asian J.* **2018**, *13*, 2923–2933. [CrossRef]

37. Ahmad, W.; Ahmad, B.; Ahmad, M.; Iqbal, Z.; Nisar, M.; Ahmad, M. In vitro inhibition of acetylcholinesterase, butyrylcholinesterase and lipoxygenase by crude extract of *Myricaria elegans* Royle. *J. Biol. Sci.* **2003**, *3*, 1046–1049.

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

39. Bilel, H.; Hamdi, N.; Zagrouba, F.; Fischmeister, C.; Bruneau, C. Terminal conjugated dienes via a ruthenium-catalyzed cross-metathesis/elimination sequence: Application to renewable resources. *Catal. Sci. Technol.* **2014**, *4*, 2064–2071. [CrossRef]

40. Ozge Karaca, E.; Imene Dehimat, Z.; Yasar, S.; Gürbüz, N.; Tebbani, D.; Çetinkaya, B.; Ozdemir, I. Ru(II)-NHC catalysed N-Alkylation of amines with alcohols under solvent-free conditions. *Inorg. Chim. Acta* **2021**, *520*, 120294. [CrossRef]

41. Çiftçi, O.; Özdemir, İ.; Çakır, O.; Demir, S. The determination of oxidative damage in heart tissue of rats caused by ruthenium(II) and gold(I) N-heterocyclic carbene complexes. *Toxicol. Ind. Health* **2011**, *27*, 735–741. [CrossRef] [PubMed]

42. Atta-ur-Rahman, W.A.T.; Nawas, S.A.; Choudhary, M.I. New Cholinesterase Inhibiting Bisbenzylisoquinoline Alkaloids from *Cocculus pendulus* . *Chem. Pharm. Bull.* **2004**, *52*, 802–806. [CrossRef] [PubMed]

43. Pizarro-Cerda, J.; Cossart, P. Microbe Profile: Listeria monocytogenes: A paradigm among intracellular bacterial pathogens. *Microbiology* **2019**, *165*, 719–721. [CrossRef] [PubMed]

44. Moore, N.M.; Flaws, M.L. Epidemiology and Pathogenesis of Pseudomonas aeruginosa Infections. *Am. Soc. Clin. Lab. Sci.* **2011**, *24*, 43–46. [CrossRef]

45. Crump, J.A.; Luby, S.P.; Mintz, E.D. The global burden of typhoid fever. *Bull. World. Health Organ.* **2004**, *82*, 346–353.

46. Molero, G.; Diez-Orejas, R.; Navarro-Garcia, F.; Monteoliva, L.; Pla, J.; Gil, C.; Sánchez-Pérez, M.; Nombela, C. *Candida albicans*: Genetics, dimorphism and pathogenicity. *Int. Microbiol.* **1998**, *1*, 95–106.

47. Ellman, G.L.; Courtney, K.D.; Andres, V.J.R.; Featherstone, R.M. A new and rapid colorimetric determination of acetyl-cholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. [CrossRef]

48. Rangkadilok, N.; Sitthimonchai, S.; Worasuttayangkurn, L.; Mahidol, C.; Ruchirawat, M.; Satayavivad, J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. *Food Chem. Toxicol.* **2007**, *45*, 328–333. [CrossRef]

49. Khan, T.A.; Koko, W.S.; Al Nasr, I.S.; Schobert, R.; Biersack, B. Activity of Fluorinated Curcuminoids against *Leishmania major* and *Toxoplasma gondii* Parasites. *Chem. Biodivers.* **2021**, *18*, e2100381. [CrossRef]

50. Re, P.; Proteggente, R.; Pannala, N.; Yang, A.; Rice-Evans, C.M.A. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]

51. Taga, M.S.; Miller, E.E.; Pratt, D.E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* **1984**, *61*, 928–931. [CrossRef]

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

53. Jelali, H.; Koko, W.; Al-Hazmy, S.M.; Mansour, L.; Al-Tamimi, J.; Deniau, E.; Sauthier, M.; Dridi, K.; Hamdi, N. Copper-Catalyzed Hydroboration of Enamides with Bis(pinacolato)diboron: Promising Agents with Antimicrobial Activities. *J. Chem.* **2022**, *2022*, 6577185. [CrossRef]

54. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [CrossRef] [PubMed]

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

56. Jentzsch, J.; Koko, W.S.; Al Nasr, I.S.; Khan, T.A.; Schobert, R.; Ersfeld, K.; Biersack, B. New Antiparasitic Bis-Naphthoquinone Derivatives. *Chem. Biodivers.* **2019**, *17*, e1900597. [CrossRef]

57. Şahin-Bölükbaşı, S.; Şahin, N. Novel Silver-NHC complexes: Synthesis and anticancer properties. *J. Organomet. Chem.* **2019**, *891*, 78–84. [CrossRef]

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