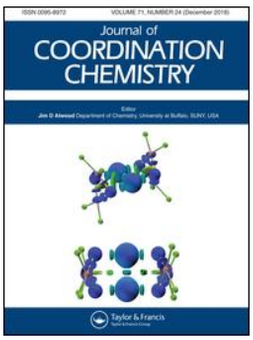

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# Anticancer, antimicrobial and antiparasitical activities of copper(I) complexes based on *N*-heterocyclic carbene (NHC) ligands bearing aryl substituents

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**ABSTRACT**

New benzimidazolium salts were synthesized as *N*-heterocyclic carbene precursors. These NHC precursors were metallated with $Cu_2O$ and CuI in acetone and water under reflux to give novel copper(I) complexes. The structures of these benzimidazolium salts and copper(I) complexes were characterized on the basis of elemental analysis, $^1$H NMR, $^{13}$C NMR, IR and LC–MS spectroscopic techniques. The (NHC)Cu(I) complexes **3–4** were tested against *MCF7* and *MDA-MB-231* cancer cells, *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* microorganisms, *Leishmania major* promastigotes and amastigotes, *Toxoplasma gondii* parasites and against Vero cell line *in vitro*. The synthesized copper NHC carbene complex **4b** (1,3-bis(2,3,4,5,6-pentamdthylbenzyl)-2,3-dihydro-1*H*-benzo[d]imidazol-2-yl)copper(I) chloride) was the most active against MCF7 cancer cells (half growth Inhibition Concentrations ($IC_{50}$) = 0.3 μg mL$^{-1}$), as well as the most potent antimicrobial against *E. coli* (inhibition zone (IZ) = 23.3 mm), MRSA (IZ = 25.5 mm) and *C. albicans* (IZ = 28.5 mm) besides its antileishmanial activities against *L. major* promastigotes and amastigotes ($IC_{50}$ < 0.04 μg mL$^{-1}$). Compound **4c** (1,3-bis(4-(tert-butyl)benzyl)-2,3-dihydro-1*H*-benzo[d]imida-zol-2-yl)copper(II) bromide) is the most potent anticancer against MDA-MB-231 cancer cells $IC_{50}$ = 0.4 μg mL$^{-1}$). Compound **4e** (5,6-dimethyl-1,3-bis(2,4,6-trimethylbenzyl)-2,3-dihydro-1*H*-benzo[d]imidazol-2-yl)copper(I) chloride) is the best suitable antitoxoplasmal

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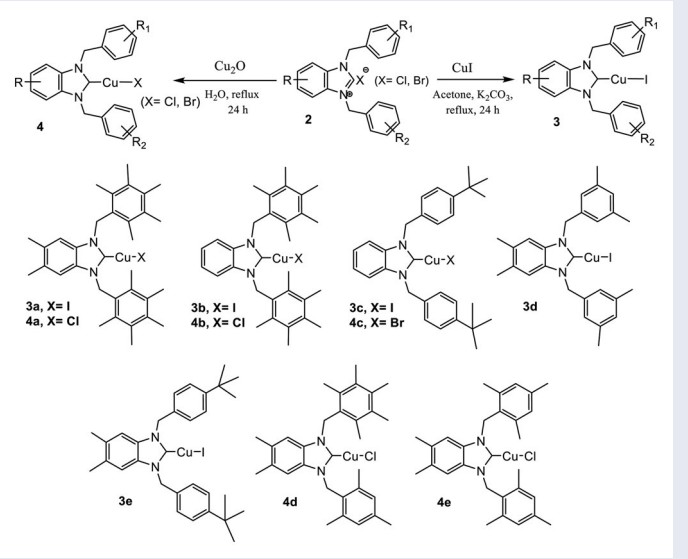
drug candidate due to its SI of 16.5. These candidates need further study to identify mode of action and drug standardization.




## 1. Introduction

The biological properties revealed for benzimidazolium salts include anticancer, antimicrobial, antiviral, anti-HIV, anti-inflammatory, antioxidant, antihypertensive and antidiabetic effects [1–15]. The substitution at carbon between two nitrogen atoms in this system usually increases the biological activities [5–7]. The activities are also enhanced upon coordination to the metal ions [16, 17].

The NHC complexes based on isoelectronic less expensive and more abundant transition metals like silver(I) and copper(I) have been also investigated because they might behave similarly from an organometallic point of view [18–23]. Indeed, gold(I) and copper(I) complexes present similar affinities toward selenium and could be considered to have similar biological mechanism [2–25]. Lazreg *et al.* [26] assumed that NHC copper complexes could generate reactive oxygen species (ROS) leading to a DNA strand break, responsible for the observed cytotoxicity. In the literature, several copper(I) complexes have been reported to exhibit half-growth inhibition concentrations ($IC_{50}$) in the micromolar (μM) range against different cancer cell lines. These copper(I) complexes are generally coordinated to phosphane and/or nitrogen ligands [27–33].

Gautier *et al.* firstly selected the [CuX(NHC)] complexes and compared their activities on different cancer cell lines such as *MCF-7*, *MCF-7R*, *LNCaP*, *HL60,* and *KB*. They demonstrated that the copper complexes stopped the cell cycle progression at the mitotic G1 phase [34]. Then, the group of Tacke synthesized copper complexes bearing benzyl-substituted NHC ligands and studied their cytotoxicity against CAKI-1 [35–40].

However, parasitical diseases are still a worldwide medical problem with particular emphasis on the neglected tropical diseases (NTDs). These diseases are perilously

dangerous for both civilians and migrants in tropical and subtropical countries. Also, the changing of the weather in many areas of the world probably leads to new parasitic infection. One of the important factors is to develop new drugs against NTDs; few pharmaceutical companies work for these drugs because they think developing countries have no ability to afford them. So, there is few numbers of drugs developed against NTDs [41–44].

The new trends are to discover drug candidates for various NTDs from cheap and renewable products. Also there are desires to direct the assistance for immunecompromised humans to some infections such as *Toxoplasma gondii* [41–44]. In the present work the synthesis and characterization of the new benzimidazolium salts (**2a–e**) and their [CuX(NHC)] complexes **3**–**4** were studied, then investigated for their activities against cancer cells *MCF7* and *MDA-MB-231*, antimicrobial (*Escherichia coli*, *MRSA* (methicillin-resistant *Staphylococcus aureus*), *Candida albicans*) and antiparasitical (*Leishmania major* and *T. gondii*). Vero cell line was tested for cytotoxicity.

## 2. Experimental

### 2.1. I-Synthesis of benzimidazolium salts

#### 2.1.1. I-1-Synthesis of benzimidazolium salts 1

A mixture of 5,6-dimethylbenzimidazole (30 mmol, 4.38 g) or benzimidazole (30 mmol, 3.54 g) and potassium hydroxide (30 mmol, 1.68 g) was dissolved in 25 mL of ethanol. The mixture was stirred for 15 min at room temperature. Then, 30 mmol of benzyl halide was added gradually. After stirring for one hour at room temperature, the mixture is refluxed for 16 h. The reaction is followed by TLC. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue obtained was dissolved in 40 mL of dichloromethane. After filtration, the solution is concentrated under reduced pressure. The yellow solid obtained is dried under vacuum [45].

***2.1.1.1. 5,6-Dimethyl-1-(2,3,4,5,6-pentamethylbenzyl)-1H-benzo[d]imidazole (1a).*** Yield: 97%, $C_{21}H_{26}N_2$, MW = 306.5, M.p. = 173 °C. ν(CN) = 1448.65 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.11 (s, 6H, $CH_{3(c,g)}$), 2.19 (s, 6H, $CH_{3(d,f)}$), 2.23 (s, 3H, $CH_{3(e)}$), 2.32 (s, 3H, $CH_{3(a)}$), 2.37 (s, 3H, $CH_{3(b)}$), 5.16 (s, 2H, $H_{1'}$), 7.20 (s, 1H, $H_7$), 7.24 (s, 1H, $H_4$), 7.50 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 16.51 ($C_{c,g}$), 16.87 ($C_{d,f}$), 17.16 ($C_e$), 20.33 ($C_a$), 20.66 ($C_b$), 44.27 ($C_{1'}$), 109.77 ($C_7$), 120.29 ($C_4$), 127.37 ($C_{5,8}$), 131.21 ($C_{4',5',6'}$), 131.97 ($C_6$), 133.38 ($C_{3',7'}$), 133.56 ($C_9$), 136.13 ($C_{2'}$), 141.10 ($C_2$).

***2.1.1.2. 1-(2,3,4,5,6-Pentamethylbenzyl)-1H-benzo[d]imidazole (1b).*** Yield: 95%, $C_{19}H_{22}N_2$, MW = 278.4, M.p. = 121 °C. ν(CN) = 1454.84 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.21 (s, 6H, $CH_{3(a,e)}$), 2.27 (s, 6H, $CH_{3(b,d)}$), 2.31 (s, 3H, $CH_{3(c)}$), 5.31 (s, 2H, $H_{1'}$), 7.35 (t, 2H, $H_{5,6}$), 7.43 (s, 1H, $H_7$), 7.56 (s, 1H, $H_4$), 7.85 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 16.55 ($C_{a,e}$), 16.89 ($C_{b,d}$), 17.19 ($C_c$), 44.42 ($C_{1'}$), 109.64 ($C_7$), 120.33 ($C_4$), 122.37 ($C_5$), 122.87 ($C_6$), 127.12 ($C_{8,9}$), 133.47 ($C_{4',5',6'}$), 133.55 ($C_{3',7'}$), 136.28 ($C_{2'}$), 141.96 ($C_2$).

***2.1.1.3. 1-(4-(Tert-Butyl)benzyl)-1H-benzo[d]imidazole (1c).*** Yield: 92%, $C_{18}H_{20}N_2$, MW = 264.4, M.p. = 123.6 °C. ν(CN) = 1457.59 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.22 (s, 9H, $CH_{3(a,b,c)}$), 5.25 (s, 2H, $H_{1'}$), 7.06 (d, 2H, $H_{3',7'}$), 7.20 (d, 2H, $H_{5,6}$), 7.29 (d, 3H, $H_{4',6',7}$), 7.77 (s, 1H, $H_4$), 7.88 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ (ppm) 31.28 ($C_{a,b,c}$), 34.60 ($C_{8'}$), 48.53 ($C_{1'}$), 110.08 ($C_7$), 120.39 ($C_4$), 122.25 ($C_5$), 123.06 ($C_6$), 125.97 ($C_{4',6'}$), 126.92 ($C_{3',7'}$), 132.46 ($C_{2',8}$), 143.20 ($C_{2,9}$), 151.40 ($C_{5'}$).

***2.1.1.4. 5,6-Dimethyl-1-(2,4,6-trimethylbenzyl)-1H-benzo[d]imidazole (1d).*** Yield: 94%, $C_{19}H_{22}N_2$, MW = 278.4, M.p. = 292.3 °C. ν(CN) = 1497.89 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.16 (s, 6H, $CH_{3(c,e)}$), 2.25 (s, 3H, $CH_{3(d)}$), 2.31 (s, 3H, $CH_{3(a)}$), 2.35 (s, 3H, $CH_{3(b)}$), 5.11 (s, 2H, $H_{1'}$), 6.88 (s, 2H, $H_{4',6'}$), 7.17 (s, 1H, $H_7$), 7.22 (s, 1H, $H_4$), 7.49 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ (ppm) 18.52 ($C_{c,e}$), 19.26 ($C_d$), 19.62 ($C_a$), 20.01 ($C_b$), 41.99 ($C_{1'}$), 108.74 ($C_7$), 119.23 ($C_4$), 126.29 ($C_5$), 128.59 ($C_{4',6'}$), 130.19 ($C_8$), 131.02 ($C_6$), 136.83 ($C_{5',9}$), 137.70 ($C_{3',7'}$), 139.86 ($C_{2'}$), 141.47 ($C_2$).

***2.1.1.5. 1-(3,5-Dimethylbenzyl)-5,6-dimethyl-1H-benzo[d]imidazole (1e).*** Yield: 93%, $C_{18}H_{20}N_2$, MW = 264.4, M.p. = 124.3 °C. ν(CN) = 1446.05 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.18 (s, 6H, $CH_{3(c,d)}$), 2.27 (s, 3H, $CH_{3(a)}$), 2.29 (s, 3H, $CH_{3(b)}$), 5.13 (s, 2H, $H_{1'}$), 6.70 (s, 2H, $H_{3',7'}$), 6.85 (s, 1H, $H_{5'}$), 7.01 (s, 1H, $H_7$), 7.51 (s, 1H, $H_4$), 7.75 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 75 MHz), δ (ppm): 20.28 ($C_a$), 20.61 ($C_b$), 21.26 ($C_{c,d}$), 48.62 ($C_{1'}$), 110.14 ($C_7$), 120.26 ($C_4$), 124.80 ($C_{3',5',7'}$), 129.78 ($C_5$), 131.11 ($C_8$), 132.22 ($C_6$), 135.70 ($C_{9,2'}$), 138.65 ($C_{4',6'}$), 142.46 ($C_2$).

### *2.1.2. I-2-Synthesis of benzimidazolium salts 2*

In a dried Schlenk tube, (1 g) of **1** and (1 eq) of benzyl halide are dissolved in 2 mL of *N*,*N*-dimethylformamide. The mixture was stirred at 70 °C for 24–48 h and the reaction was monitored by TLC. At the end of the reaction, the reaction mixture was diluted with 30 mL of diethyl ether to precipitate the product. After filtration, the white solid obtained was dried under vacuum and then recrystallized from a mixture of DCM-ether solvents (1:3) for further purification [46].

***2.1.2.1. 5,6-Dimethyl-1,3-bis(2,3,4,5,6-pentamethylbenzyl)-1H-benzo[d]imidazol-3-ium chloride (2a).*** Yield: 96%, $C_{33}H_{43}N_2Cl$, MW = 503.2, M.p. = 307.7 °C. ν(CN) = 1440.99 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.22 (s, 30H, $CH_3$), 2.28 (s, 6H, $CH_{3(a,b)}$), 5.80 (s, 4H, $H_{1',1''}$), 7.11 (s, 2H, $H_{4,7}$), 9.85 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 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 ($C_{4;7}$), 125.58 ($C_{8;9}$), 130.42 ($C_{4';5';6';4'';5'';6''}$), 133.50 ($C_{5;6}$), 133.74 ($C_{3';7';3'';7''}$), 136.88 ($C_{2';2''}$), 141.35 ($C_2$).

***2.1.2.2. 1,3-Bis(2,3,4,5,6-pentamethylbenzyl)-1H-benzo[d]imidazol-3-ium chloride (2b).*** Yield: 92%, $C_{31}H_{39}N_2Cl$, MW = 475.1, M.p. = 210.6 °C. ν(CN)= 1553.91 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), 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, $H_{1',1''}$), 7.20 (d, 2H, $H_{5,6}$), 7.34 (d, 2H, $H_{4,7}$), 10.34 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), 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 ($C_{2';2''}$), 143.0 ($C_2$).

*2.1.2.3. 1,3-Bis(4-(tert-butyl)benzyl)-1H-benzo[d]imidazol-3-ium bromide (2c).* Yield: 89%, $C_{29}H_{35}N_2Br$, MW = 491.5, M.p. = 296.5 °C. ν(CN) = 1562.86 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.20 (s, 18H, $CH_3$), 5.75 (s, 4H, $H_{1',1''}$), 7.33 (d, 4H, $H_{4',6',4'',6''}$), 7.39 (d, 4H, $H_{3';7'3'',7''}$), 7.46 (d, 2H, $H_{5;6}$), 7.55 (d, 2H, $H_{4;7}$), 11.83 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 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 ($C_{2';2''}$), 143,0 ($C_2$), 152.46 ($C_{5';5''}$).

*2.1.2.4. 5,6-Dimethyl-1-(2,3,4,5,6-pentamethylbenzyl)-3-(2,4,6-trimethylbenzyl)-1H-benzo[d]imidazol-3-ium chloride (2d).* Yield: 94%, $C_{31}H_{39}N_2Cl$, MW = 475.1, M.p. = 272.2 °C. ν(CN) = 1567.83 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.17 (s, 6H, $CH_{3(a,b)}$), 2.21 (s, 24H, $CH_{3(c,d,e,f,g,h,i,j)}$), 5.68 (s, 2H, $H_{1'}$), 5.80 (s, 2H, $H_{1''}$), 6.83 (s, 2H, $H_{4'';6''}$), 7.04 (s, 1H, $H_4$), 7.20 (s, 1H, $H_7$), 10.41 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 16.96 ($C_{d,f}$), 17.09 ($C_{c,g}$), 17.30 ($C_e$), 20.17 ($C_{a,b}$), 20.75 ($C_{h,j}$), 21.03 ($C_i$), 47.46 ($C_{1'}$), 47.82 ($C_{1''}$), 113.09 ($C_7$), 113.54 ($C_4$), 125.19 ($C_8$), 125.75 ($C_9$), 129.96 ($C_{4'';6''}$), 130.3 ($C_{5'}$), 133.52 ($C_{4';6'}$), 133.92 ($C_{3';7'}$), 136.83 ($C_{5''}$), 136.90 ($C_{2''}$), 137.15 ($C_{3'',7''}$), 137.88 ($C_{5;6}$), 139.32 ($C_{2'}$), 142.17 ($C_2$).

*2.1.2.5. 5,6-Dimethyl-1,3-bis(2,4,6-trimethylbenzyl)-1H-benzo[d]imidazol-3-ium chloride (2e).* Yield: 96%, $C_{29}H_{35}N_2Cl$, MW = 447.1, M.p. = 245.6 °C. ν(CN) = 1567.86 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.23 (s, 6H, $CH_{3(a,b)}$), 2.29 (s, 18H, $CH_{3(c,c',d,d',e,e')}$), 5.79 (s, 4H, $H_{1',1''}$), 6.91 (s, 6H, $H_{arom}$), 10.92 (s, 1H, $H_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 20.21 ($C_{c,e,c',e'}$), 20.74 ($C_{a,b}$), 21.06 ($C_{d,d'}$), 47.23 ($C_{1';1''}$), 113.29 ($C_{4,7}$), 125.40 ($C_{8,9}$), 130.07 ($C_{4',6';4'',6''}$), 130.25 ($C_{5',5''}$), 136.88 ($C_{2',2''}$), 137.91 ($C_{3',7';3'';7''}$), 139.56 ($C_{5,6}$), 142.70 ($C_2$).

## 2.2. II-Synthesis of copper complexes

### 2.2.1. II-1-Synthesis of copper complexes CuI 3

A standard Schlenk was loaded with benzimidazolium salts **2** (1 mmol), copper iodide (1 mmol, 0.19 g) and $K_2CO_3$ (2 mmol, 0.276 g). The resulting mixture was heated under reflux in acetone at 60 °C under argon for 24 h. After this time, the mixture was filtered on a silica column, eluting with dichloromethane. The product obtained was concentrated with a rotary evaporator and dried under vacuum affording white complexes **3a–3e** [47].

*2.2.1.1. (5,6-Dimethyl-1,3-bis(2,3,4,5,6-pentamethylbenzyl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)copper(I) iodide (3a).* Yield: 68%, $C_{33}H_{42}N_2ICu$, MW = 657.2, M.p. = 265.6 °C. ν(CN) = 1448.51 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.12 (s, 12H, $CH_{3(c,g,c,g'')}$), 2.16 (s, 12H, $CH_{3(d,f,d',f'')}$), 2.19 (s, 6H, $CH_{3(e,e')}$), 2.21 (s, 6H, $CH_{3(a,b)}$), 5.39 (s, 4H, $H_{1',1''}$), 6.86 (s, 2H, $H_{4,7}$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 16.10 ($C_{c,g,c',g'}$), 16.25 ($C_{d,f,d',f'}$), 16.36 ($C_{e,e'}$), 19.46 ($C_{a,b}$), 47.80 ($C_{1';1''}$), 110.85 ($C_{4;7}$), 126.35 ($C_{8;9}$), 131.79 ($C_{4';6';4'';6''}$), 131.9 ($C_{5';5''}$), 132.09 ($C_{5;6}$), 132.91 ($C_{3';7';3'';7''}$), 135.67 ($C_{2';2''}$), 184.99 ($C_2$). Anal. Calc. for

$C_{33}H_{42}N_2ICu$ (%): C, 60.31; H, 6.44; N, 4.26. Found (%): C, 60.39; H, 6.52; N, 4.34. HR-MS(ESI), (*m/z*) = 529.2614 $[M\text{-}I]^+$ (Calc. for $C_{33}H_{42}N_2Cu$: 529.2644).

#### 2.2.1.2. (1,3-Bis(2,3,4,5,6-pentamethylbenzyl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)copper(I) iodide (3b).

Yield: 72%, $C_{31}H_{38}N_2ICu$, MW = 629.1, M.p. = 245.3 °C. ν(CN) = 1419.02 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.13 (s, 12H, $CH_{3(a,e,a',e')}$), 2.17 (s, 12H, $CH_{3(b,d,b',d')}$), 2.22 (s, 6H, $CH_{3(c,c')}$), 5.47 (s, 4H, $H_{1',1''}$), 7.08 (d, 2H, $H_{5;6}$), 7.15 (d, 2H, $H_{4,7}$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 16.11 ($C_{a,e,a',e'}$), 16.26 ($C_{b,d,b',d'}$), 16.38 ($C_{c,c'}$), 48.13 ($C_{1';1''}$), 110.61 ($C_{4;7}$), 122.76 ($C_{5;6}$), 126.13 ($C_{5';5''}$), 132.08 ($C_{4';6';4'';6''}$), 132.98 ($C_{3';7';3'';7''}$), 133.22 ($C_{8;9}$), 135.79 ($C_{2';2''}$), 186.25 ($C_2$). Anal. Calc. for $C_{31}H_{38}N_2ICu$ (%): C, 59.19; H, 6.09; N, 4.45. Found (%): C, 59.25; H, 6.17; N, 4.56. HR-MS(ESI), (*m/z*): 501.2398 $[M\text{-}I]^+$ (Calc. for $C_{31}H_{38}N_2Cu$: 501.2331).

#### 2.2.1.3. (1,3-Bis(4-(tert-butyl)benzyl)-2,3-dihydro-1H-benzo[d]imida-zol-2-yl)copper(I) iodide (3c).

Yield: 66%, $C_{29}H_{34}N_2ICu$, MW = 601.1, M.p. = 208.1 °C. ν(CN) = 1476.56 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$, 400 MHz), δ (ppm): 1.20 (s, 18H, $CH_3$), 5.71 (s, 4H, $H_{1',1''}$), 7.28 (s, 6H, $H_{4',6',4'',6'';5,6}$), 7.39 (d, 4H, $H_{3',7',3'',7''}$), 7.59 (d, 2H, $H_{4,7}$). $^{13}C$ NMR (DMSO-$d_6$, 100 MHz), δ (ppm): 36.23 ($C_{a,b,c,a',b',c'}$), 39.43 ($C_{8',8''}$), 55.85 ($C_{1'',1''}$), 117.18 ($C_{4,7}$), 128.74 ($C_{5,6}$), 130.57 ($C_{4',6',4'',6''}$), 132.52 ($C_{3',7',3'',7''}$), 138.63 ($C_{8,9}$), 138.71 ($C_{2',2''}$), 155.47 ($C_{5',5''}$), 194.20 ($C_2$). Anal. Calc. for $C_{29}H_{34}N_2ICu$ (%): C, 57.95; H, 5.70; N, 4.66. Found (%): C, 58.03; H, 5.76; N, 4.74. HR-MS(ESI), (*m/z*): 473.2044 $[M\text{-}I]^+$ (Calc. for $C_{29}H_{34}N_2Cu$: 473.2018).

#### 2.2.1.4. (1,3-Bis(3,5-dimethylbenzyl)-5,6-dimethyl-2,3-dihydro-1H-benzo[d]imidazol-2-yl)copper(I) iodide (3d).

Yield: 63%, $C_{27}H_{30}N_2ICu$, MW = 573, M.p. = 232.5 °C. ν(CN) = 1440.29 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.25 (s, 12H, $CH_{3(c,d,c',d')}$), 2.28 (s, 6H, $CH_{3(a,b)}$), 5.50 (s, 4H, $H_{1',1''}$), 6.92 (s, 6H, $H_{3',5',7',3'',5'',7''}$), 7.06 (s, 2H, $H_{4,7}$). $^{13}C$ NMR ($CDCl_3$, 75 MHz), δ (ppm): 19.40 ($C_{a,b}$), 20.28 ($C_{c,d,c',d'}$), 51.42 ($C_{1',1''}$), 110.96 ($C_{4,7}$), 124.29 ($C_{3',7',3'',7''}$), 128.96 ($C_{5',5''}$), 131.40 ($C_{8,9}$), 132.32 ($C_{5,6}$), 134.27 ($C_{2',2''}$), 137.58 ($C_{4',6',4'',6''}$), 181.58 ($C_2$). Anal. Calc. for $C_{27}H_{30}N_2ICu$ (%): C, 56.60; H, 5.28; N, 4.89. Found (%): C, 56.68; H, 5.38; N, 4.98. HR-MS(ESI), (*m/z*): 383.2472 $[M\text{-}I]^+$ (Calc. for $C_{27}H_{30}N_2Cu$: 383.2443).

#### 2.2.1.5. (1,3-Bis(4-(tert-butyl)benzyl)-5,6-dimethyl-2,3-dihydro-1H-benzo[d]imidazol-2-yl)copper(I) iodide (3e).

Yield: 65%, $C_{31}H_{38}N_2ICu$, MW = 629.1, M.p. = 225.4 °C. ν(CN) = 1365.35 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 1.21 (s, 18H, $CH_{3(c,d,e,c',d',e')}$), 2.13 (s, 6H, $CH_{3(a;b)}$), 4.97 (s, 4H, $H_{1',1''}$), 6.62 (s, 2H, $H_{4,7}$), 7.20 (d, 4H, $H_{3',7',3'',7''}$), 7.24 (d, 4H, $H_{4',6',4'',6''}$). $^{13}C$ NMR ($CDCl_3$, 75 MHz), δ (ppm): 18.92 ($C_{a;b}$), 30.29 ($C_{c,d,e,c',d',e'}$), 33.47 ($C_{8',8''}$), 43.49 ($C_{1',1''}$), 108.40 ($C_{4,7}$), 124.61 ($C_{4',6',4'',6''}$), 126.10 ($C_{3',7',3'',7''}$), 126.51 ($C_{8,9}$), 128.34 ($C_{5,6}$), 132.62 ($C_{2',2''}$), 143.39 ($C_{5',5''}$), 180.90 ($C_2$). Anal. Calc. for $C_{31}H_{38}N_2ICu$ (%): C, 59.19; H, 6.09; N, 4.45. Found (%): C, 59.25; H, 6.17; N, 4.56. HR-MS(ESI): *m/z* = 439.3090 $[M\text{-}I]^+$ (Calc. for $C_{31}H_{38}N_2Cu$: 439.3069).

### 2.2.2. II-2-Synthesis of copper complexes CuX 4

A round bottom flask was charged with benzimidazolium salt **2** (2 mmol), copper oxide (1.3 mmol, 0.186 g) and deionized water (6 mL). The mixture was heated under

reflux for 24 h. After this time, the reaction mixture was allowed to cool to room temperature, then diluted with DCM and the organic fraction was extracted. The organic extract was dried with $MgSO_4$ and concentrated under reduced pressure. The product obtained was purified by chromatography on a silica column, eluting with dichloromethane to give the desired product in the form of an off-white solid [48].

***2.2.2.1. (5,6-Dimethyl-1,3-bis(2,3,4,5,6-pentamethylbenzyl)-2,3-dihy-dro-1H-benzo[d]imidazol-2-yl)copper(I) chloride (4a).*** Yield: 70%, $C_{33}H_{42}N_2ClCu$, MW = 565.7, M.p. = 265.6 °C. ν(CN) = 1447.07 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.12 (s, 12H, $CH_{3(c,g,c',g')}$), 2.16 (s, 18H, $CH_{3(d,e,f,d',e',f')}$), 2.21 (s, 6H, $CH_{3(a,b)}$), 5.39 (s, 4H, $H_{1',1''}$), 6.82 (s, 2H, $H_{4,7}$).$^{13}C$ NMR($CDCl_3$, 75 MHz), δ (ppm): 15.99 ($C_{c,g,c',g'}$), 16.21 ($C_{d,e,f,d',e',f'}$), 19.45 ($C_{a,b}$), 48,0 ($C_{1';1''}$), 110.77 ($C_{4,7}$), 126.49 ($C_{8,9}$), 131.78 ($C_{4',6';4'';6''}$), 131.83 ($C_{5';5''}$), 132.13 ($C_{5,6}$), 132.71 ($C_{3',7',3'',7''}$), 135.38 ($C_{2',2''}$), 182.69 ($C_2$). Anal. Calc. for $C_{33}H_{42}N_2ClCu$ (%): C, 70.06; H, 7.48; N, 4.95. Found (%): C, 70.15; H, 7.59; N, 5.05. HR-MS(ESI), (*m/z*): 529.2619 $[M\text{-}Cl]^+$ (Calc. for $C_{33}H_{42}N_2Cu$: 529.2644).

***2.2.2.2. (1,3-Bis(2,3,4,5,6-pentamdthylbenzyl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl) copper(I) chloride (4b).*** Yield: 74%, $C_{31}H_{38}N_2ClCu$, MW = 537.7, M.p. = 238.8 °C. ν(CN) = 1435.31 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.22 (s, 12H, $CH_{3(a,e,a',e')}$), 2.25 (s, 12H, $CH_{3(b,d,b',d')}$), 2.30 (s, 6H, $CH_{3(c,c')}$), 5.48 (s, 4H, $H_{1',1''}$), 7.04 (d, 2H, $H_{5,6}$), 7.10 (d, 2H, $H_{4,7}$). $^{13}C$ NMR ($CDCl_3$, 75 MHz), δ (ppm): 17.05 ($C_{a,e,a',e'}$), 17.26 ($C_{b,c,d,b',c',d'}$), 49.37 ($C_{1',1''}$), 111.57 ($C_{4,7}$), 123.72 ($C_{5,6}$), 127.26 ($C_{5',5''}$), 133.13 ($C_{4',6',4'',6''}$), 133.84 ($C_{8,9}$), 134.28 ($C_{3',7',3'',7''}$), 136.58 ($C_{2';2''}$), 186.03 ($C_2$). Anal. Calc. for $C_{31}H_{38}N_2ClCu$ (%): C, 69.25; H, 7.12; N, 5.21. Found (%): C, 69.37; H, 7.19; N, 5.34. HR-MS(ESI), (*m/z*): 501.2391 $[M\text{-}Cl]^+$ (Calc. for $C_{31}H_{38}N_2Cu$: 501.2331).

***2.2.2.3. (1,3-Bis(4-(tert-butyl)benzyl)-2,3-dihydro-1H-benzo[d]imida-zol-2-yl)copper(I) bromide (4c).*** Yield: 63%, $C_{29}H_{34}N_2BrCu$, MW = 554.1, M.p. = 208.1 °C. ν(CN) = 1479.23 cm $^1$. $^1H$ NMR ($CDCl_3$, 300 MHz), δ (ppm): 1.21 (s, 18H, $CH_3$), 5.55 (s, 4H, $H_{1',1''}$), 7.19-7.34 (m, 12H, $H_{arom}$). $^{13}C$ NMR ($CDCl_3$, 75 MHz), δ (ppm): 30.23 ($C_{a,b,c,a',b',c'}$), 33.56 ($C_{8',8''}$), 51.58 ($C_{1',1''}$), 110.96 ($C_{4,7}$), 123.0 ($C_{5,6}$), 124.96 ($C_{4',6',4'',6''}$), 126.27 ($C_{3',7',3'',7''}$), 130.99 ($C_{8,9}$), 132.79 ($C_{2',2''}$), 150.49 ($C_{5',5''}$), 184.60 ($C_2$). Anal. Calc. for $C_{29}H_{34}N_2BrCu$ (%): C, 62.87; H, 6.19; N, 5.06. Found (%): C, 62.96; H, 6.26; N, 5.16. HR-MS(ESI), (*m/z*): 473.2043 $[M\text{-}Br]^+$ (calc. for $C_{29}H_{34}N_2Cu$: 473.2018).

***2.2.2.4. (5,6-Dimethyl-1-(2,3,4,5,6-pentamethylbenzyl)-3-(2,4,6-trimethylbenzyl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)copper(I) chloride (4d).*** Yield: 68%, $C_{31}H_{38}N_2ClCu$, MW = 537.7, M.p. = 231.3 °C. ν(CN) = 1450.56 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.22 (s, 15H, $CH_{3(c,d,e,f,g)}$), 2.27 (s, 6H, $CH_{3(h,j)}$), 2.30 (s, 6H, $CH_{3(a,b)}$), 2.32 (s, 3H, $CH_{3(i)}$), 5.38 (s, 2H, $H_{1'}$), 5.54 (s, 2H, $H_{1''}$), 6.67 (s, 1H, $H_7$), 6.89 (s, 2H, $H_{4'';6''}$), 7.10 (s, 1H, $H_4$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), δ (ppm): 17.10 ($C_{c,g}$), 17.15 ($C_{d,f}$), 17.10 ($C_e$), 20.42 ($C_a$), 20.51 ($C_b$), 20.66 ($C_{h,j}$), 21.04 ($C_i$), 47.69 ($C_{1'}$), 49.58 ($C_{1''}$), 111.35 ($C_7$), 112.18 ($C_4$), 127.13 ($C_{4'',6''}$), 127.62 ($C_{8,9}$), 129.91 ($C_{4',5',6'}$), 132.98 ($C_{5,6}$), 134.14 ($C_{3',7'}$), 136.86 ($C_{5''}$), 137.55 ($C_{3'',7''}$), 138.57 ($C_{2',2''}$), 183.86 ($C_2$)). Anal. Calc. for $C_{31}H_{38}N_2ClCu$ (%): C, 69.32; H, 7.17; N, 5.30. Found (%): C, 69.2; H, 7.12; N, 5.21. HRMS (ESI), (*m/z*): [M + Cl], found 571.4046.

$C_{31}H_{38}N_2CuCl_2$ requires 571.1708; $[M\text{-}Cl]^+$, found 501.2463. $C_{31}H_{38}N_2Cu$ requires 501.2331; found 439.3121. $C_{31}H_{39}N_2$ requires 439.3113.

***2.2.2.5. (5,6-Dimethyl-1,3-bis(2,4,6-trimethylbenzyl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)copper(I) chloride (4e).*** Yield: 72%, $C_{29}H_{34}N_2ClCu$, MW = 509.6, M.p. = 229.3 °C. $\nu$(CN) = 1465.85 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz), $\delta$ (ppm): 2.25 (s, 18H, $CH_{3(c,c',d,d',e,e')}$), 2.33 (s, 6H, $CH_{3(a;b)}$), 5.45 (s, 4H, $H_{1',1''}$), 6.85 (s, 2H, $H_{4,7}$), 6.94 (s, 4H, $H_{4',6',4'',6''}$). $^{13}C$ NMR ($CDCl_3$, 100 MHz), $\delta$ (ppm): 20.50 ($C_{c,d,e,c',d',e'}$), 21.11 ($C_{a,b}$), 48.28 ($C_{1';1''}$), 111.80 ($C_{4;7}$), 127.27 ($C_{4';6';4'';6''}$), 130.11 ($C_{5,6,8,9}$), 133.12 ($C_{5';5''}$), 137.52 ($C_{3',7';3'',7''}$), 139.04 ($C_{2;2''}$), 183.78 ($C_2$). Anal. Calc. for $C_{29}H_{34}N_2ClCu$ (%): C, 68.41; H, 6.77; N, 5.61. Found (%): C, 68.35; H, 6.72; N, 5.50. HRMS (ESI), (*m/z*): $[M\text{-}Cl]^+$, found 473.1990. $C_{29}H_{34}N_2Cu$ requires 473.2018; found 411.2756. $C_{29}H_{35}N_2$ requires 411.2800.

## 2.3. Biological activities

### 2.3.1. Anticancer cytotoxicity activities

3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was used to evaluate the efficacy of the compounds against *MDA-MB-231* and *MCF7* cells as carried out previously [23]. The cells were cultured into 96-well plates (Corning, USA) at a density of $5 \times 10^5$ cells per well in 200 µL medium. After 24 h, cells were treated with different concentrations (10, 5, 2.5, 1 µg $mL^{-1}$) of compounds. After 48 h incubation period, 20 µL MTT solution was added to each well and cells were further incubated for 2 h at 37 °C. At the end, the media were removed by aspiration and then 200 µL 0.1% HCl-MeOH was added to dissolve formazan crystals. The OD value was read at 490 nm on a microplate reader (Thermo MULTISCAN FC, China). MeOH-treated cells were used as controls (negative). The number of replications for the anticancer cytotoxic studies was three. The compounds were screened colorimetrically using MTT cell assay.

The relative cell viability was calculated using the following formula:

$$\text{Relative cell viability } (\%) = (\text{OD treated}/\text{OD control}) \times 100\ (\%)$$

## 2.4. Disk diffusion method

Nutrient agar plates were used for culturing *E. coli* (ATCC® 10418) and *MRSA* (ATCC®3359) while potato dextrose agar of (HiMedia, India) was used for culturing and *C. albicans* (ATCC® 90028) for 24 h at 35 °C. The cultures were obtained from the American Type Culture Collection (ATCC). The disc diffusion technique was used to assess the antimicrobial activities [22]. Sterile saline solution (0.9%) was used for microbes and the turbidity was adjusted to 0.5 OD value using a spectrophotometer (Labomed Inc., USA). Sterilized cotton swab was used to put the inoculum in to the surface of agar plates. Sterile blank disks (6-mm) were loaded with 10 µL of compounds stock solution (10 mg/mL) giving a concentration of 50 µg/disc. Commercial tetracycline discs (30 µg per disc) were used as positive controls and methanol as a negative control for comparison. The petri-plates were incubated at 35 °C for 24 h. The inhibition zones (IZ) were assessed as diameters (mm) produced by the compounds on the test microoganisms.

### 2.5. Leishmania major *cell isolation, culture conditions, and assays*

*Leishmania major* promastigotes were collected from indoor male patient and maintained at 26 °C in Schneider's Drosophila medium (Invitrogen, USA) supplied by 10% inactivated fetal bovine serum (FBS, Invitrogen, USA) with antibiotics using tissue culture flask followed by weekly transfers, and finally cryopreserved in liquid nitrogen with amount of $3 \times 10^6$ parasite $mL^{-1}$ [49].

For assessing the compounds activity. Promastigotes from logarithmic-phase cultured in phenol red-free RPMI 1640 medium (Invitrogen, USA) with 10% FBS were suspended on 96-well plates to yield $10^6$ cells $mL^{-1}$ (200 µL/well). Compounds and reference drug AmB were added using different concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14, and 0.04 µg $mL^{-1}$). DMSO (1%) was used as negative control. Plates were incubated at 26 °C for 72 h. MTT colorimetric assay was used for assessing the number of viable promastigotes. The samples were analyzed using an ELISA reader at 570 nm. Obtained $IC_{50}$ values resulted from three repeated experiments [49].

For evaluating compounds against *L. major* amastigotes, peritoneal macrophages from female BALB/c mice (6–8 weeks of age) were collected [50], then $5 \times 10^4$ cells/well were seeded on 96-well plates in phenol red-free RPMI 1640 medium with 10% FBS for 4 h at 37 °C in 5% $CO_2$ to enhance the adhesion of the cells. PBS was used for washing after the media discarded. About 200 µL containing *L. major* promastigotes solution was added per well. For allowing infection with amastigotes the plates were incubated for 24 h at 37 °C in humidified 5% $CO_2$. Then, PBS was used for washing the infected macrophages and covered with fresh phenol red-free RPMI 1640 medium containing compounds and AmB at different concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14, and 0.04 µg $mL^{-1}$) and incubated at 37 °C in humidified 5% $CO_2$ for 72 h. DMSO (1%) was used as negative control. The percentage of infected macrophages were evaluated microscopically after removing medium, washing, fixation, Giemsa staining. Obtained $IC_{50}$ values resulted from three repeated experiments [49].

### 2.6. Toxoplasma gondii *cell line, culture conditions, and assay*

Vero cells (ATCC® CCL81™, USA) were used for the cultivation of *T. gondii* tachyzoites of the RH strain obtained from State Key Laboratory for Agrobiotechnology, China Agricultural University, Bejing, China. Complete RPMI 1640 medium with 10% FBS inactivated was used for culturing the cells in a humidified 5% $CO_2$ at 37 °C. 96-Well plates ($5 \times 10^3$ cells/well in 200 µL RPMI 1640 medium) were used for the cultivation of the Vero cells and then incubated at 37 °C and 5% $CO_2$ for 24 h, followed by removal of medium and washing the cells with PBS. Then, RPMI 1640 medium with 2% FBS containing tachyzoites (RH strain) of *T. gondii* at a parasites:Vero cells ratio of 5:1 were added. After incubation at 37 °C and 5% $CO_2$ for 5 h, cells were washed with PBS and then treated with compounds and ATO reference drug with different concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14, and 0.04 µg $mL^{-1}$) while DMSO (1%) was used as negative control. 1% toluidine blue was used for staining the cells after 72 h incubation at 37 °C and 5% $CO_2$ followed by washing with PBS and fixation in 10% formalin. Inverted photomicroscope was for the determination

of the infection index (number of cells infected from 200 cells tested) of *T. gondii*. Then % inhibition was calculated as:

$$\text{Inhibition } (\%) = (\text{I Control} - \text{I Experimental})/(\text{I Control}) \times 100$$

Then effects of test compounds on parasite growth were expressed as $IC_{50}$ values, from three repeated experiments [44, 51].

### 2.7. In vitro *cytotoxicity assay*

For assessing compounds cytotoxicity, MTT assay was used against Vero cells as previously described against cancer cells according to OECD guidelines [52]. Complete RPMI 1640 medium with 10% FBS was used for culturing Vero cells in 96-well plates (5 $10^3$ cells/well/200 µL) then incubated for 24 h at 5% $CO_2$ and 37 °C. Cells were washed with PBS and treated with test compounds for 72 h at varying concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14, and 0.04 µg $mL^{-1}$) in medium with 10% FBS. Some cells were treated with medium in 2% FBS to be kept as negative control. Then the media was removed by aspiration and 50 µL of RPMI 1640 medium containing 14 µL MTT (5 mg $mL^{-1}$) was added followed by 4 h incubation. The supernatant was removed again and 200 µL DMSO was added to each well with continuous shaking for 15 min. A FLUOstar OPTIMA spectrophotometer was applied for colorimetric analysis ($\lambda = 540$ nm). Cytotoxic effects were expressed by $CC_{50}$ values (concentration that caused a 50% reduction in viable cells). Three different experiments were repeated for $CC_{50}$ calculation [44].

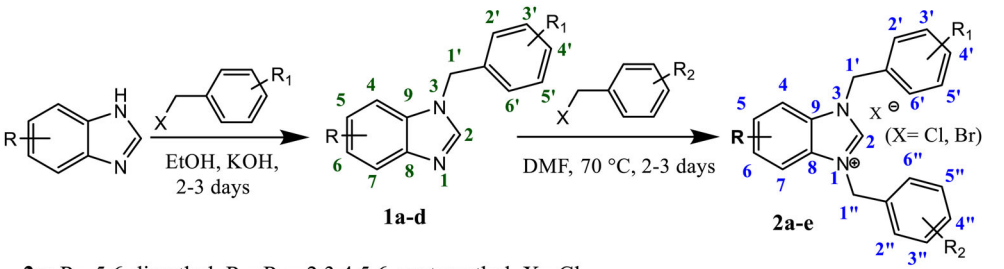
## 3. Results and discussion

### 3.1. Synthesis and characterization

*N*-heterocyclic carbene ligands have been proven very popular in the last 20 years. Conjugate reduction of α,β-unsaturated ketones and esters, the hydrosilylation of ketones, the cyclopropanation of terminal alkenes, as well as olefinations, carbene transfer reactions, aziridination of olefins and methylenation of aldehydes are among some examples of the uses of Cu-NHC complexes (specifically (IPr) CuCl) in modern catalysis. Finally, these catalysts are air- and moisture-stable and they can be used as precursors to synthesize more air-sensitive complexes [53].

All the benzimidazolium salts as NHC precursors were already prepared similarly to the published procedures [54, 55]. As shown in Scheme 1, benzimidazolium salts **2a–e** were synthesized in good yields by quaternization of **1** in DMF at 70 °C for 03 days with the corresponding arylchlorides. The benzimidazolium salts **2a–e** are stable to air and moisture, both in the solid state and in solution. They were characterized by $^1$H-NMR, $^{13}C\{^1H\}$ NMR, IR and elemental analysis techniques.

The structures of the benzimidazolium salts **2** can be easily confirmed by the $^1$H NMR spectroscopic data. The characteristic carbonic protons (NCHN) are located at 9.85, 10.34, 11.83, 10.41, 10.92, 11.51, 11.42, and 11.63 ppm, respectively. The corresponding methylene protons appear as singlets at 5.8, 5.84, 5.75, 5.68, 5.79, 5.68, 5.65, and 5.77 ppm, respectively, which are comparable to the reported values [56–58]. As




**2a:** R= 5,6-dimethyl, $R_1$=$R_2$= 2,3,4,5,6-pentamethyl, X= Cl
**2b:** R= H, $R_1$=$R_2$= 2,3,4,5,6-pentamethyl, X= Cl
**2c:** R= H, $R_1$=$R_2$= 4-tertbutyl, X= Br
**2d:** R= 5,6-dimethyl, $R_1$= 2,3,4,5,6-pentamethyl, $R_2$= 2,4,6-trimethyl, X= Cl
**2e:** R= 5,6-dimethyl, $R_1$=$R_2$= 2,4,6-trimethyl, X= Br

**Scheme 1.** Protocol synthesis benzimidazolium salts **2a–e**.

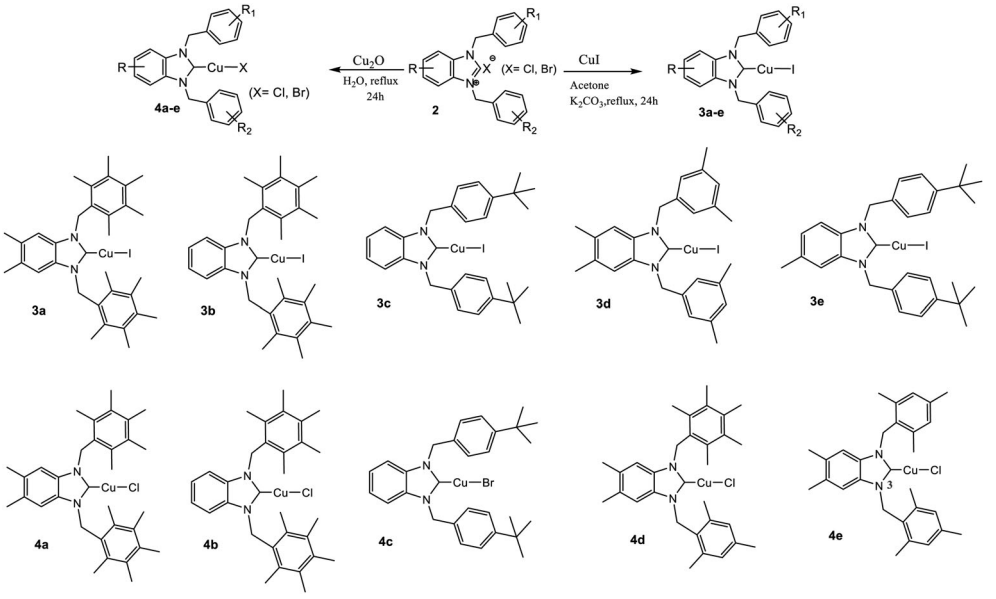
expected, the absence of pro-carbenic protons can be observed upon coordination of the benzimidazolium salts with the copper(I), confirming formation of the NHC–Cu(I) complexes **3**–**4**. In the $^{13}$C NMR spectra, the signals for the carbene carbon atoms of salts **2a–e** appear at 141.3, 143.0, 143.0, 143, 142.1, 142.07, 141.6, 141.6, 141.8, and 141.83 ppm, respectively, which are consistent with signals for other NHC–Cu(I) complexes [59]. The [CuCl(NHC)] complexes **4** were already synthesized following the Nolan–Cazin procedure using $Cu_2O$ as metal precursor for 24 h in water under reflux and the appropriate benzimidazole. These metal(II) complexes were obtained as white solids in 63–74% yield.

Complexes **3a-e** were obtained by reacting benzimidazolium salts **2a–c** with $K_2CO_3$ in acetone under reflux for 24 h in acetone in the presence of CuI in good yields (Scheme 2).

In recent years, copper *N*-heterocyclic carbene complexes (Cu-NHCs) have found applications as catalysts [60] and as NHC transfer reagents [61]. In the clear majority of these cases, the copper is present in oxidation state Cu(I), where the soft–soft acid–-base interaction, Cu(I)–C, results in a highly stable complex. Also, when the NMR of the solution in the NMR tube was taken again days later, same NMR spectrum had been seen.

The elemental analysis data of [CuCl(NHC)] complexes **3–4** are in agreement with the theoretical values for the synthesized complexes. The benzylic –$CH_2$– proton signals $H_{1',1''}$ for complex [CuCl(NHC)] **2e** as representative were observed at 5.45 ppm, while the aromatic protons appeared at $\delta$ between 6.85 and 7.26 ppm. The carbene carbon signal of **2e** was observed at 183.7 ppm in the $^{13}$C NMR spectrum, while the $C_{1',1''}$ carbon signals were at $\delta$ 48.2 ppm. The mass spectrum of **2e** gave the most prominent peaks at $m/z = 279$ and 411, respectively.

The $^1$H NMR spectra of the Cu-NHC complexes (I) **3–4** showed less intense and downfield shifted benzimidazole signals as compared to the free ligands. In $^{13}$C NMR spectra of the complexes, a downfield shift in C = N resonance of the ligands upon complexation indicates the binding of benzimidazolium salts to copper through the imine nitrogen atom. The aromatic carbons of benzene ring resonate between 112 and 141 ppm. In case of the ligands the parallel ring carbons behave as equivalent but




**Scheme 2.** Protocol synthesis of [CuCl(NHC)] complexes **3–4**.

**Table 1.** Anticancer activity of Cu-NHC complexes **3–4**.

| | Anticancer activity, IC50 (in μM) | |
|---|---|---|
| Cu–NHC complexes (II) **3–4** | MCF7 | MDA-MB-231 |
| **3a** | 0.943 | 0.806 |
| **3b** | NA | NA |
| **3c** | 1.181 | 1.364 |
| **3d** | NA | NA |
| **3e** | NA | NA |
| **4a** | 0.795 | 1.095 |
| **4b** | 0.595 | 1.153 |
| **4c** | 1.155 | 0.794 |
| **4d** | NA | NA |
| **4e** | NA | NA |
| *Tetracycline* | 0.697 | |

NA, not active.

in the complexes they showed separate signals. The methyl peak in the Cu-NHC complexes (I) **3–4** is observed around 16 ppm. These results are in agreement with the data of other such complexes [58, 62–67].

## 3.2. Biological evaluation

### 3.2.1. Anticancer evaluation

Table 1 indicates that the five compounds were highly efficient and active against the two types of cancer cells investigated in this study. For *MCF7*, the five compounds (**4b**, **4a**, **3a**, **4c,** and **3c**) had $IC_{50}$ ($IC_{50}$ is the lethal concentration required to kill 50% of the population) values less than 1 μg $mL^{-1}$ with an $IC_{50}$ = 0.3, 0.4, 0.6, 0.6, and 0.7 μg $mL^{-1}$, respectively; for *MDA-MB-231*, the same compounds had $IC_{50}$ values of

**Table 2.** Antimicrobial profile of synthesized derivatives Cu–NHC complexes (I) **3**–**4**[a].

| | Antimicrobial activity (50 μg/disc) | | |
|---|---|---|---|
| Cu–NHC complexes (I) **3–4** | *E. coli* | *MRSA* | *C. albicans* |
| **3a** | 18 ± 2 | 15 ± 0.5 | 16.0 ± 00 |
| **3b** | 16 ± 1 | 15 ± 00 | 28.0 ± 00 |
| **3c** | 16 ± 0.6 | 18.0 ± 00 | 10.0 ± 00 |
| **3d** | 8 ± 0.6 | 10.±1.0 | 0.00 ± 00 |
| **3e** | 12 ± 00 | 13 ± 00 | 14 ± 00 |
| **4a** | 21.0 ± 1 | 22 ± 2 | 25.0 ± 00 |
| **4b** | 23 ± 1 | 25 ± 1 | 28 ± 1 |
| **4c** | 21 ± 0.6 | 23.0 ± 001 | 28.0 ± 00 |
| **4d** | 18 ± 1 | 8 ± 0.5 | 0.0 ± 00 |
| **4e** | 13 ± 00 | 12 ± 00 | 14 ± 00 |
| **2a** | 12 | 11 | 10 |
| **2b** | 13 | 11 | 12 |
| **2c** | 11 | 10 | 12 |
| **2d** | 12 | 11 | 12 |
| **2e** | 13 | 12 | 13 |
| *Tetracycline* | 20 | 16 | – |
| *Fluconazole* | – | – | 24 |

Values are mean value ± standard deviation of three different replicates.
[a]The concentration was 50 μg.

0.6, 0.6, 0.5, 0.4, and 0.8 μg $mL^{-1}$, respectively. The other compounds showed no activity against the cancer cells. These results agree with the previous results of Kizrak *et al.* [68], who studied different compounds of silver and gold *N*-heterocyclic carbene complexes against a number of human cancer cells and finally stated that these types of research provide exciting opportunities for discovering new types of metallodrugs. We can conclude our results enhance the discovery of new anticancer drugs based on metal complex compounds (Table 1).

### 3.2.2. Antimicrobial activities

A simple inspection of Table 2 indicates that the highest antibacterial activity against *E. coli* was reported by **4b** with inhibition zone (IZ) 23.3 mm followed by **4a** and **4c** with IZ = 21 mm for all of them. Also, **4b** is the most active against MRSA with IZ = 25.5 mm followed by **4c** (IZ = 23 mm) and **4a** (IZ = 22.5 mm). **4b** also proved the best antifungal activity against *C. albicans* (IZ = 28.5 mm) followed by **3b** and **4c** (IZ = 28 mm) and then **4a** (IZ = 25 mm). NHC metals particularly silver synthesized compounds as well as copper derivatives were found to have potent antibacterial activities [69, 70]. Our results here agree with this finding. These results have shown that benzimidazolium salts **2a–e** precursors have lower antibacterial activity when compared with the Cu–NHC complexes (I) **3–4**, which have promising results, with good activity against bacteria and fungi, although the mechanism of antimicrobial activity is not known.

### 3.3.3. Antileishmanial activities

Table 3 shows all the compounds except **4e** revealed antileishmanial activity against both *L. major* amastigotes and promastigotes *in vitro* with $IC_{50}$ lower than 9 μg $mL^{-1}$. Seven compounds, **3c**, **3e**, **4a**, **4b**, **4c,** and **4d**, possess $IC_{50}$ lower than 0.4 μg $mL^{-1}$ against *L. major* promastigotes while four of them (**3c**, **4b**, **4c,** and **4d**) showed $IC_{50}$

**Table 3.** Antileishmanial activity of Cu–NHC complexes against *L. major* promastigotoes and amastigotes.

| Cu–NHC complexes (II) **3–4** | $CC_{50}$ of Vero cells at μM | Amastigote $IC_{50}$ at μM | Promastigotes $IC_{50}$ at μM | Amastigote SI | Promastigote SI |
|---|---|---|---|---|---|
| **3a** | 1.035 | 1.978 | 12.173 | 0.52 | 0.08 |
| **3b** | 6.358 | 6.358 | 12.875 | 0.83 | 0.42 |
| **3c** | <0.665 | <0.665 | <0.665 | – | – |
| **3d** | 1.431 | 2.967 | 4.712 | 0.48 | 0.30 |
| **3e** | <0.636 | 6.199 | <0.636 | – | – |
| **4a** | <0.707 | 4.949 | <0.707 | – | – |
| **4 b** | 1.692 | <0.744 | <0.744 | >2 | >2 |
| **4c** | <0.722 | <0.722 | <0.722 | – | – |
| **4d** | 0.948 | <0.744 | <0.744 | >1 | >1 |
| **4e** | 125.588 | 56.907 | 147.763 | 2.15 | 0.86 |
| **AmB** | 7.4 ± 2.64 | 0.46 ± 0.07 | 0.78 ± 0.09 | 16 | 9.49 |

Amphotericin B is an antileishmania reference drug.

**Table 4.** Antitoxopolasmal activity of Cu-NHC complexes (I) **3–4** against *T. gondii*.

| Cu–NHC complexes (I) **3–4** | $CC_{50}$ of Vero cells at μM | Antitoxoplasma $IC_{50}$ at μM | SI |
|---|---|---|---|
| **3a** | 1.035 | <0.608 | >1 |
| **3b** | 5.404 | 11.444 | 0.47 |
| **3c** | <0.665 | <0.665 | – |
| **3d** | 1.431 | 4.188 | 0.34 |
| **3e** | <0.636 | <0.636 | – |
| **4a** | <0.707 | <0.707 | – |
| **4 b** | 1.692 | 4.835 | 0.35 |
| **4c** | <0.722 | <0.722 | – |
| **4d** | 0.948 | 1.562 | 0.62 |
| **4e** | 126.374 | 7.653 | 16.51 |
| ATO | 9.3 ± 2.08 | 0.09 ± 0.02 | 103.33 |

ATO, Atovaquone is an antitoxoplasma reference drug.

lower than 0.4 μg $mL^{-1}$ against *L. major* amastigotes. Compounds **4b** and **4d** are less toxic and their SI over 1 for both *L. major* promastigotes and amastigotes. In recent studies a group of metal-based complexes were found of strong antileishmanial activities against *L. major* promastigotes and *Trypanosoma cruzi* due to their interference with DNA formation [70], also some gold complexes against *L. infantum* promastigotes and amastigotes [71]. These results support our results here for Cu–NHC complexes as antileishmanial against *L. major* promastigotes and amastigotes *in vitro*.

### *3.3.4. Antitoxoplasmal activities*

Table 4 indicates that all the compounds possess good antitoxoplasmal activity against *T. gondii in vitro* with $IC_{50}$ lower than 7.5 μg $mL^{-1}$. Five of them (**3a**, **3**c, **3e**, **4a,** and **4d**) have $IC_{50}$ lower than 0.4 μg $mL^{-1}$, followed by **4d**, **3d**, **4b**, **4e,** and **3 b** with $IC_{50}$ = 0.84, 2.4, 2.6, 3.9, and 7.2 μg $mL^{-1}$, respectively. The table also shows **4e** possesses very good SI with 16.51, which indicates the safety of this compound as an antitoxoplasmal drug candidate. However, NHC–carbene metal complexes had not previously been investigated against *T. gondii*, but some of their derivatives mainly of silver and gold were found of good antiparasitical activities against some of apicomplexes protozoa such as *Plasmodium* spp. [72]. This finding agrees with our results for NHC-copper complexes against *T. gondii*.

## 4. Conclusions

In the present investigation, the synthesized copper NHC–carbene complex **4b** was found to be the most active against *MCF7* cancer cells, as well as the most potent antimicrobial against *E. coli*, *MRSA* and *C. albicans* besides its antileishmanial activities against *L. major* promastigotes and amastigotes. Compound **4c** is the most potent anticancer against *MDA-MB-231* cancer cells. Compound **4e** is the most suitable anti-toxoplasmal drug candidate from all investigated compounds due to its SI of 16.5. Further pharmacological investigations based on the mode of action and drug standardization are highly recommended for these drug candidates.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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