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SILVER(I) COMPLEXES BEARING AMINE-FUNCTIONALIZED N-HETEROCYCLIC CARBENES: SYNTHESIS, ANTIMICROBIAL AND THEORETICAL STUDIES

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Abstract – In this study, a series of amine functionalized benzimidazolium salts and their silver(I) N-heterocyclic carbene complexes were synthesized and characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy and mass spectrometry. All the compounds were evaluated for their antibacterial and antifungal activities. They showed antibacterial activity with MIC values in the range of 12.5-50 μg/mL and antifungal activity with MIC values in the range of

25-100 μg/mL. Notably, compounds **3d** and **3e** exerted antibacterial activity against all tested Gram negative and Gram positive bacteria including *Staphylococcus aureus* MRSA strain with a quite low MIC value of 12.5 μg/mL. Besides these, computational study was applied to understand the biochemical activity analyzes of Ag complexes **3a-e**. The findings suggest that there are components **3a** and **3b** with potential drug properties for future advance researches.

INTRODUCTION

Infectious diseases caused by bacteria and fungi are extremely common worldwide with serious consequences on human population and also causes heavy economic burden. For treatment and sometimes prevention of infectious diseases antimicrobial agents have vital role. Antibiotics with different structure and mechanisms of action have been used for decades and obviously have saved life of hundreds of millions. Ability of bacteria to gain resistance against antibiotics and increasing number of resistant bacteria to antibiotics create a big threat for public health. Although several generations of antibiotics are produced, the resistance problem stays as a significant obstacle to fight against bacterial diseases resulting in higher morbidity and eventually mortality rates. In addition to higher morbidity and mortality antibiotic resistance is associated with higher economic burden worldwide.[1]

Recently, several plant-derived natural compounds have been tested for their potential antibacterial properties and some are suggested be considered as an alternative to conventional antibiotics.[2] WHO report on the antibacterial clinical and preclinical pipeline indicates that only few antibiotics have been approved in recent years and 26 of 43 were reported to be active against the WHO priority pathogens.[3] These would not be sufficient solve the problem of antimicrobial resistance. Thus new strategies are needed to develop and test new compounds to overcome the danger of antimicrobial resistance.

N-Heterocyclic carbenes (NHCs) and their metal complexes have been intensively studied over the last two decades for medical applications.[4-6] Especially, Ag(I) and Au(I)-NHC complexes displayed significant antimicrobial and anticancer activity.[7-11] The first example of silver(I)-NHC complex was synthesized by the reaction of silver triflate with free carbene, which derived from imidazolium salt by Arduengo et al. in 1993.[12] The isolation of free carbene requires harsh inert conditions that limits the synthesis of silver complexes. To overcome this limitation, a facile synthesis of silver(I)-NHC complexes by deprotonation of azolium salts with silver oxide were developed by Lin et al. in 1998.[13] The *in situ* deprotonation by a basic silver source such as silver(I) oxide, silver(I) acetate and silver(I) carbonate has become the most common used method for the synthesis of silver(I)-NHC complexes.[13-15] The antimicrobial properties of silver NHC complexes were first reported by Youngs et al. in 2004.[16] Since then, silver(I)-NHC complexes have attracted great interest in numerous areas of research such as

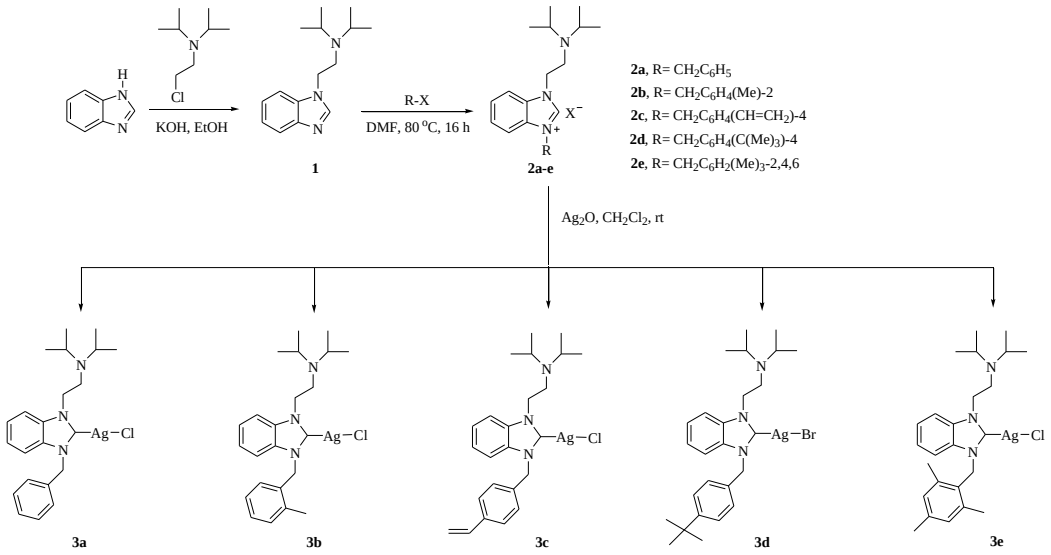
medicinal chemistry, coordination chemistry, catalysis and material sciences.[17-20] In particular, its diverse applications in medicinal chemistry are well studied.[21-24] A wide range of silver complexes of NHCs based on imidazole, triazole, benzimidazole and imidazolidine scaffolds were synthesized and explored as efficient antibacterial, antifungal and anticancer agents.[25-29] Also, Ag(I)-NHC complexes have been evaluated for their antibiofilm activities, and found to be effective against biofilms of pathogenic bacteria.[30,31] In addition, some Ag(I)-NHC complexes have exhibited inhibitory activity against the metabolic enzymes.[32,33] Benzimidazolium salts are the most widely used precursors for the synthesis of Ag(I)-NHC complexes, and have antimicrobial, antitumor, anti-inflammatory and anthelmintic properties.[34-40] A great number of Ag(I) and Au(I) complexes bearing mono- and bis-benzimidazole ligands with antimicrobial and anticancer potentials were reported.[41-46]

In a recent study, we reported the synthesis of diisopropylamine-functionalized NHC-Pd(II) PEPPSI complexes, which demonstrated good inhibition effects against carbonic anhydrase I, II (hCAs I and II), and α-glycosidase enzymes.[47] In another study, reported by Özdemir, Ag(I) and Au(I) complexes of diisopropylamine-substituted benzimidazolium salts exhibited good anticancer activity against human cell lines, namely brain (SHSY5Y), colon (HTC 116), and liver (HEP3B).[48] In this study, we present the synthesis, antibacterial and antifungal activities of the diisopropylamine-substituted benzimidazolium salts and their respective Ag(I)-NHC complexes. Furthermore, biochemical activity analyzes of related complex structures were investigated on the basis of quantum chemical parameters.[49] In the meantime, the toxic properties of metal complex structures against the living body were investigated and compounds that could show potential drug properties were predicted by using Data Warrior 5.5.0.[50]

RESULTS AND DISCUSSION

Synthesis of silver(I) complexes

In this study, the benzimidazolium salts **2a-e** as N-heterocyclic carbene precursors were prepared via the two-step N-alkylation process as depicted in Scheme 1. Compound **1**, 1-(2-diisopropylaminoethyl)benzimidazole was obtained by the reaction of benzimidazole with 2-diisopropylaminoethyl chloride in the presence of KOH in ethanol at room temperature. The reaction of 1-(2-diisopropylaminoethyl)benzimidazole **1** with variety alkyl halides in *N*,*N*-dimethylformamide at 80 ^{o}C afforded the benzimidazolium salts **2a-e**, which were purified by recrystallization from ethanol/diethyl ether. In the ^{1}H NMR spectra of **2c**, NC*H*N proton appeared as a singlet at 11.61 ppm and benzylic protons appeared as a singlet at 5.88 ppm. The N*C*HN carbon resonance of **2c** was observed at 144.10 ppm in the ^{13}C NMR spectra. The appearances of these downfield signals indicate the formation of **2c**. The other benzimidazolium salts used in this study (**2a, 2b, 2d** and **2e**) were previously reported by our group.[51,52]



Scheme 1. The synthesis and structures of silver complexes

The Ag(I)-NHC complexes **3a-e** were prepared via the *in situ* deprotonation of benzimidazolium salts by Ag_2O according to the general method described by Wang and Lin.[13] Treatment of the benzimidazolium salts with Ag_2O in dichloromethane at room temperature for 24 hours in the dark afforded the expected silver complexes Ag(I)-NHC **3a-e** (Scheme 1). The Ag(I)-NHC complexes **3a-e** were obtained in high yields as white solids, soluble in halogenated solvents. These complexes are stable in air, but are light sensitive. The structures of silver(I) complexes were determined by ^{1}H NMR, ^{13}C NMR, IR spectroscopy and mass spectrometry, which support the proposed structures. The formation of Ag(I)-NHC complexes **3a-d** were confirmed by the absence of the resonance signals of benzimidazolium (NC*H*N) proton in the region 10.72-11.61 ppm in the ^{1}H NMR spectra and benzimidazolium (N*C*N) carbon in the region 144.10-152.40 ppm in ^{13}C NMR spectra. In the Ag(I)-NHC complexes **3a-d**, the resonances for carbene carbon were not observed, which has also been mentioned in the literature and given as a reason for the fluxional behavior of the Ag-NHCs.[53]

Antibacterial activities

The antimicrobial activities of ten compounds (**2a–e** and **3a-e**) were assessed against four Gram negative (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*), three Gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Staphylococcus aureus* MRSA), and two yeast (*Candida glabrata* and *Candida albicans*) strains at different concentrations. Results were shown in Table 1. All compounds were found to exhibit significant antibacterial activities against strains tested and seven compounds against fungi strains at the concentrations below 100 µg/mL. Overall antibacterial efficacy of compounds **2c** and **2d** was higher against Gram positive strains than Gram negative strains while compounds **3a-e** exerted similar inhibitory effects against both Gram positive and

Gram negative strains and this effect was higher than the inhibitory effect of compounds **2a-e**. Among bacterial strains, *Staphylococcus aureus* was the most susceptible strain to all compounds at MIC of 25 and 12.5 μg/mL. Compounds **3d** bearing 4-*tert*-butylbenzyl group and **3e** bearing 2,4,6-trimethylbenzyl group showed significantly higher inhibitory effects against all bacterial strains with the MIC values of 12.5 μg/mL. Although compound **2c** having 4-vinylbenzyl group had a high MIC values (above 25 μg/mL) against Gram negative bacteria, it had low MIC values against Gram positive bacteria (12.5 μg/mL). For yeast strains, the lowest MIC values of compounds **3b, 3c** and **3e** which may be considered as the best action were detected against both strains of yeast.[54] These results clearly revealed that introducing the 2-diisopropylaminoethyl into the benzimidazole scaffold increased the antibacterial and antifungal activity significantly, when compared with similar previously reported benzimidazolium salts bearing substituted benzyl groups and their silver complexes.[36,55-57]

Table 1. Mininal inhibitory concentrations (μg/mL) of **2a-e** and **3a-e**

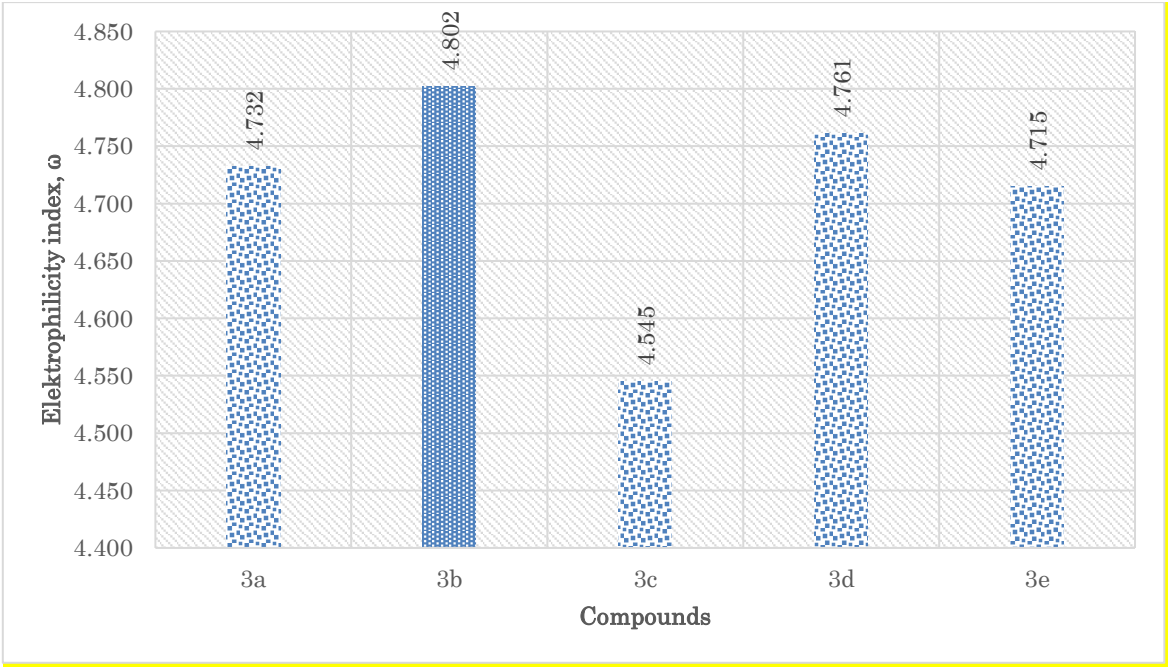
Compound	Gram negative bacteria				Gram positive bacteria			Fungi	
	Escherichia Coli	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Staphylococcus aureus MRSA*	*Candida glabrata*	*Candida albicans*
2a	25	50	50	50	25	50	50	100	100
2b	25	25	25	25	25	25	50	50	50
2c	25	50	50	50	12.5	12.5	12.5	50	50
2d	50	50	25	25	12.5	25	25	50	50
2e	12.5	25	25	25	25	25	50	50	100
3a	25	25	25	25	25	25	25	100	25
3b	12.5	50	12.5	12.5	25	12.5	12.5	25	25
3c	12.5	12.5	25	25	25	25	25	25	25
3d	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25	50
3e	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25	25
Gentamicin	3.12		3.12	1.56	1.56	1.56			
Sefazolin	1.56	1.56		1.56					
Amikacin	1.56	1.56	3.12	1.56	1.56				
Itrakonazol								1.56	3.12
Teikoplanin					1.56	1.56	1.56		
Meropenem				1.56	1.56		1.56		

Results of computational study

Biological activity is the result of selected molecular species interacting with a biological entity. The human organism in clinical studies and experimental animals (*in vivo*) or experimental models (*in vitro*) in preclinical studies represent a biological entity. Besides, the biological activity depends on the nature of the compound, namely the differences in its chemical structure and physicochemical properties. In this context, biological activities can be identified and determined at the organism, organ/tissue and cellular and molecular levels. If the molecule is found to have several activities at different levels, the activities of additional levels can be considered as the cause. Approaches to evaluate compounds with activity, conceptually, important similarities and features in molecular structure are responsible for the same biological activity. However, structure and activity can be obtained in many different ways/sources, and it is difficult to construct general molecular representations that capture structure-activity relationships for various sets of molecules. The structure-activity relationship has been developed in the current study for the Ag compounds, which are conceptually handled with the help of a single descriptor; that is, on the basis of the electrophilicity index, the biological activity and also the toxicity were tried to be estimated *in silico*.

After the structural and energy optimization of Ag complexes by using Gaussian 09 program, their quantum chemical parameters include HOMO and LUMO eigenvalues, dipole moments (DM), global hardness (η), chemical potential (μ), and electrophilicity index (ω) were calculated. Based on the calculations, the electrophilicity index values, which are an important parameter in the estimation of the effectiveness of the chemical structures of the related complexes on biological systems,[49,58-60] were determined indirectly with implemented of the direct calculated parameters. Moreover, a possible descriptor of biological activity, the electrophilicity index supports or even strengthens interpretations on the biological activity of the compounds in the present study. Therefore, compound **3b**, which has methyl group in the second position of the phenyl ring, is the most effective with a value of 4.802. Secondly, compound **3d**, in which the bromine atom, not the chlorine, is located in the Ag complex and the *tert*-butyl group in the 4th position of the phenyl ring takes the second order with the value of 4.761. The compound **3a** ranks 3rd with 4.732 of electrophilic index value. The compound **3e**, on the other hand, contains methyl groups at 2, 4 and 6 positions of the phenyl ring, and with a value of 4.715, it also took the 4th place due to the steric effect. The last compound **3c** takes the last place because the electron-withdrawing vinyl group at 4 position of the phenyl ring reduces the reactivity of the structure. Table 2 clearly states this situation.

Table 2. Biochemical activity analyzes of the Ag complexes **3a-e**



In summary, groups such as methyl and *tert*-butyl groups provide chemical reactivity to Ag complexes because they are electron donating groups, but it is not preferred that too many or all *meta* and *para* positions are attached to electron donating groups. Within this complex homologous series, the compound **3b** structure including the methyl group attached to the 2nd position of the phenyl ring is most suitable. On the other side, Data Warrior 5.5.0 system was utilized to examine the efficacy of metal complexes as well as the toxicity of such structures in living systems. Toxicity risk analyzes cover mutagenic, tumorigenic, irritant and reproductive activity properties of each complex. Moreover, drug likeness values and drug score values were also determined to predict the drug potential of these complexes. The toxicity estimation process relies on a pre-calculated set of structural parts that, if encountered in the already drawn structure, leads to toxicity warnings. These parts lists have been generated by rigorous breakdown of all compounds in the Registry of Toxic Effects of Chemicals (RTECS) database known to be active in a particular toxicity class (eg mutagenicity). During fragmentation, any molecule was first cut at each rotatable bond, yielding a series of core fragments. These, in turn, were used to reconstruct all possible larger fragments that were a substructure of the original molecule. A background research process then determined the frequency of occurrence of any part (core and built part) within all compounds in that toxicity class. As a result of the parameters estimated according to the toxicity risk analysis shown in Table 3, the drug properties and drug scores of the investigated compounds were calculated. It is observed that **3a** and **3b** compounds, respectively, with drug properties of 2,400 and 1.903, are among the

compounds discussed. Moreover, since they are the two related structures with the highest drug score values, it would be beneficial to further or organize future studies on these compounds.

Table 3. The Toxicity Risk Assessment of the potential compounds (NM: non-mutagenic (-); NT: non-tumorigenic (-); NI: non-irritant (-); LR: low risk for reproductive efficacy (-); Drug likeness indicates a positive drug likeness value; Drug score: Numerical values close to drug score 1 used to assess the overall potential of the compound to qualify for a given drug are desirable values.)

Comp.	Mutagenic	Tumorigenic	Irritant	Reproductive effective	Druglikeness	Drug score
3a	-	-	+	-	2.400	0.276
3b	-	-	+	-	1.903	0.235
3c	-	-	+	-	-0.276	0.153
3d	-	-	+	-	-0.720	0.153
3e	-	-	+	-	-2.795	0.099

CONCLUSION

In summary, a group of benzimidazolium salts containing diisopropylaminoethyl and benzyl moieties, and their Ag(I) complexes were successfully synthesized and characterized using spectroscopic methods. These compounds were tested against some Gram positive and Gram negative bacteria and two fungal strains. All compounds showed antibacterial activity with MIC values in the range of 12.5-50 μg/mL and and antifungal activity with MIC values in the range of 25-100 μg/mL. Notably, compounds **3d** and **3e** exerted antibacterial activity against all tested Gram negative and Gram positive bacteria including *Staphylococcus aureus* MRSA strain with a quite low MIC value of 12.5 μg/mL. The MIC value of a compound has substantial importance for an antibiotic therapy. However this test is not sufficient for a compound to act in the organism due to several factors. In addition to bacteriostatic tests, bactericidal effects should be assessed. On the basis of computational study, the relationship between the chemical structures of the compounds and their biological activities has been tried to be estimated. Toxicity risk

analyzes, which is one of the undesirable side effects of its biological activities, were also investigated in order to guide future studies.

EXPERIMENTAL

Materials and methods

All reactions for the preparation of the benzimidazolium salts and Ag(I)-NHC complexes were carried out under argon in flame-dried glassware using standard Schlenk techniques. Chemicals and solvents were purchased from Sigma-Aldrich. The solvents used were purified by distillation over the drying agents indicated and were transferred under argon. Benzimidazolium salts (**2a, 2b, 2d and 2e**) and silver complex (**3e**) were prepared according to the literatures.[48,51,52] ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian AS 400 Merkur spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C) in $CDCl_3$ and DMSO-d_6 with tetramethylsilane as an internal reference. ^{1}H peaks were labeled as singlet (s), doublet (d), triplet (t), septet (sept) and multiplet (m). Chemical shifts and coupling constants (*J* values) were reported in ppm and in Hz, respectively. FT-IR spectra were recorded on ATR unit in the range 400-4000 cm^{-1} on PerkinElmer Spectrum 100. The mass spectrometric analysis was performed at the Thermo Scientific Exactive Plus Benchtop Full-Scan Orbitrap Mass Spectrometer LC-MS/MS analyzer. Melting points were measured in open capillary tubes with Stuart SMP 40 melting point apparatus and uncorrected.

General procedure for the preparation of the benzimidazolium salts, 2

To a solution of 1-(2-diisopropylaminoethyl)benzimidazole (10.00 mmol) in DMF (5 mL), alkyl chloride (10.15 mmol) was added. The reaction mixture was stirred at room temperature for 2 h and heated at 80 ^{o}C for 18 h. After reaction completed, the reaction mixture was cooled to room temperature. Et_2O (10 mL) was added to obtain a white crystalline solid, which was filtered off. The solid was washed with Et_2O and dried under vacuum. The crude product was recrystallized from EtOH/Et_2O.

1-(2-Diisopropylaminoethyl)-3-(4-vinylbenzyl)benzimidazolium chloride, 2c

Yield: 3.54 g, 89%; mp 127-128 ºC; IR, $\upsilon_{(NCN)}$ = 1558.9 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 11.61 (s, 1H, NC*H*N), 7.72-7.37 (m, 8H, NC_6H_4N and $CH_2C_6H_4$(vinyl)-4), 6.70-6.63 (m, 1H, Ar-C*H*=CH_2), 5.88 (s, 2H, C*H*$_2$-Ar) 5.74 and 5.28 (d, 2H, *J* = 12.0 Hz, Ar-CH=C*H*$_2$), 4.59-4.57 (m, 2H, C*H*$_2$$CH_2N(Pr^i)_2$), 3.03-3.00 (m, 2H, NC*H*$(CH_3)_2$), 2.98-2.94 (m, 2H, CH_2C*H*$_2$$N(Pr^i)_2$), 0.78 (d, 12H, *J* = 4.0 Hz, NCH(C*H*$_3$)$_2$). ^{13}C NMR (δ, $CDCl_3$): 144.10 (N*C*HN), 138.42, 135.85, 132.34, 131.68, 130.93, 128.76, 126.97, 126.82, 126.74, 115.13, 113.58 and 113.12 (N*C*$_6$H$_4$N and $CH_2C_6H_4$(*C*H=*C*H$_2$)-4), 50.94 (*C*H$_2$-Ar), 47.73

(CH$_2$CH$_2$N(Pr^i)$_2$), 47.41 (NCH(CH$_3$)$_2$), 44.09 (CH$_2$$CH_2$N(Pr^i)$_2$), 20.66 (NCH(CH$_3$)$_2$). HR-AM (*m/z*): Calcd for $C_{24}H_{32}N_3$ [M-Cl]$^+$: 362.2596, found: 362.2553.

General procedure for the preparation of silver(I)-NHC complexes, 3

A solution of the appropriate benzimidazolium salt (1.12 mmol), Ag_2O (0.56 mmol) and activated molecular sieves 4Å in CH_2Cl_2 (25 mL) was stirred for 24 h at room temperature under argon, and the Schlenk was covered with aluminum foil. The reaction mixture was filtered through celite, and the solvent was removed under vacuum. The crude product was recrystallized from CH_2Cl_2/hexane at room temperature.

Chloro[1-(2-diisopropylaminoethyl)-3-benzylbenzimidazol-2-ylidene]silver(I), 3a

Yield: 0.38 g, 72%, mp 185-186 ºC, IR: $\nu_{(NCN)}$ = 1477.3 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 7.45-7.18 (m, 9H, NC$_6$$H_4$N and CH$_2C_6$$H_5$), 5.53 (s, 2H, C$H_2C_6H_5$), 4.31 (t, 2H, *J* = 8.0 Hz, CH_2CH$_2$N(Pr^i)$_2$), 2.95 (sept, 2H, *J* = 8.0 Hz, NCH(CH$_3$)$_2$), 2.85 (t, 2H, *J* = 8.0 Hz, CH$_2$CH_2N(Pr^i)$_2$), 0.86 (d, 12H, *J* = 8.0 Hz, NCH(CH_3)$_2$). ^{13}C NMR (δ, $CDCl_3$): 134.89, 134.35, 133.46, 129.06, 128.52, 127.18, 124.13, 124.08, 112.09 and 111.68 (NC$_6$H$_4$N and CH$_2$$C_6H_5$), 53.45 ($CH_2$Ar), 50.68 ($CH_2CH_2$N(Pr^i)$_2$), 48.84 (N$C$H(CH$_3$)$_2$), 45.50 (CH$_2$$CH_2$N(Pr^i)$_2$), 20.94 (NCH($CH_3$)$_2$). HR-AM (*m/z*): Calcd for $C_{22}H_{30}N_3$ [M-Ag-Cl+H]$^+$: 336.2440, found: 336.2395.

Chloro[1-(2-diisopropylaminoethyl)-3-(2-methylbenzyl)benzimidazol-2-ylidene]silver(I), 3b

Yield: 0.41 g, 74%, mp 143-144 ºC, IR: $\nu_{(NCN)}$ = 1478.4 cm^{-1}. ^{1}H NMR (δ, DMSO-d_6): 7.85-6.67 (m, 8H, NC$_6$$H_4$N and CH$_2C_6$$H_4$(CH$_3$)-4), 5.70 (s, 2H, C$H_2$Ar), 4.43 (t, 2H, *J* = 8.0 Hz, CH_2CH$_2$N(Pr^i)$_2$), 2.96 (sept, 2H, *J* = 4.0 Hz, NCH(CH$_3$)$_2$), 2.87 (t, 2H, *J* = 8.0 Hz, CH$_2$CH_2N(Pr^i)$_2$), 2.38 (s, 3H, CH$_2$C$_6$H$_4$(CH_3)-4), 0.81 (d, 12H, *J* = 4.0 Hz, NCH(CH_3)$_2$). ^{13}C NMR (δ, DMSO-d_6): 136.05, 134.94, 134.03, 134.00, 130.90, 128.15, 126.49, 124.38, 112.76 and 112.70 (NC$_6$H$_4$N and CH$_2$$C_6H_5$), 50.17 ($CH_2$Ar), 50.09 ($CH_2CH_2$N(Pr^i)$_2$), 48.12 (N$C$H(CH$_3$)$_2$), 44.87 (CH$_2$$CH_2$N(Pr^i)$_2$), 21.12 (NCH($CH_3$)$_2$), 19.56 (s, 3H, CH$_2C_6H_4$($CH_3$)-4). HR-AM (*m/z*): Calcd for $C_{23}H_{32}N_3$ [M-Ag-Cl+H]$^+$: 350.2596, found: 350.2550.

Chloro[1-(2-diisopropylaminoethyl)-3-(4-vinylbenzyl)benzimidazol-2-ylidene]silver(I), 3c

Yield: 0.44 g, 78%; mp 202-203 ºC; IR, $\upsilon_{(NCN)}$ = 1477.5 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 7.45-7.15 (m, 8H, NC$_6$$H_4$N and CH$_2C_6$$H_4$(vinyl)-4), 6.59 (dd, 1H, *J* = 12.0 Hz, Ar-CH=CH$_2$), 5.65 and 5.18 (d, 2H, *J* = 16.0 Hz, Ar-CH=CH_2), 5.52 (s, 2H, CH_2-Ar), 4.30 (t, 2H, *J* = 8.0 Hz, CH_2CH$_2$N(Pr^i)$_2$), 2.95 (sept, 2H, *J* = 8.0 Hz, NCH(CH$_3$)$_2$), 2.84 (t, 2H, *J* = 8.0 Hz, CH$_2$CH_2N(Pr^i)$_2$), 0.86 (d, 12H, *J* = 8.0 Hz, NCH(CH_3)$_2$). ^{13}C

NMR (δ, $CDCl_3$): 137.84, 135.99, 134.35, 134.31, 133.41, 127.45, 126.82, 124.14, 124.09, 114.72, 112.10, 111.69 (NC_6H_4N and $CH_2C_6H_4(CH{=}CH_2)$-4), 53.27 (CH_2-Ar), 50.67 ($CH_2CH_2N(Pr^i)_2$), 48.84 ($NCH(CH_3)_2$), 45.49 ($CH_2CH_2N(Pr^i)_2$), 20.94 ($NCH(CH_3)_2$). HR-AM (*m/z*): Calcd for $C_{24}H_{32}N_3$ $[M\text{-}Ag\text{-}Cl+H]^+$: 362.2596, found: 362.2548.

Bromo[1-(2-diisopropylaminoethyl)-3-(4-*tert*-butylbenzyl)benzimidazol-2-ylidene]silver(I), 3d

Yield: 0.46 g, 71%, mp 192-193 °C, IR: $\nu_{(NCN)}$ = 1477.1 cm^{-1}. ^1H NMR (δ, $CDCl_3$): 7.44-7.14 (m, 8H, NC_6H_4N and $CH_2C_6H_4(CH_3)_3$-4), 5.50 (s, 2H, CH_2Ar), 4.30 (t, 2H, *J* = 8.0 Hz, $CH_2CH_2N(Pr^i)_2$), 2.95 (sept, 2H, *J* = 4.0 Hz, $NCH(CH_3)_2$), 2.84 (t, 2H, *J* = 8.0 Hz, $CH_2CH_2N(Pr^i)_2$), 1.20 (s, 9H, $CH_2C_6H_4(CH_3)_3$-4), 0.86 (d, 12H, *J* = 8.0 Hz, $NCH(CH_3)_2$). ^{13}C NMR (δ, $CDCl_3$): 134.29, 133.55, 131.99, 127.01, 125.95, 124.04, 123.98, 112.14 and 111.58 (NC_6H_4N and $CH_2C_6H_4(CH_3)_3$-4), 53.01 (CH_2Ar), 50.64 ($CH_2CH_2N(Pr^i)_2$), 48.90 ($NCH(CH_3)_2$), 45.51 ($CH_2CH_2N(Pr^i)_2$), 34.59 ($CH_2C_6H_4C(CH_3)_3$-4), 31.25 ($CH_2C_6H_4C(CH_3)_3$-4), 20.99 ($NCH(CH_3)_2$). HR-AM (*m/z*): Calcd for $C_{26}H_{38}N_3$ $[M\text{-}Ag\text{-}Br+H]^+$: 392.3066, found: 392.3015.

Antimicrobial assays

The benzimidazolium salts **2a-e** and their Ag(I) complexes **3a-e** were tested at a concentration of 1000-1.56 µg/mL against the standard culture collections of the most frequently isolated strains among the society depending on the suggestions of Clinical Laboratory Standards Institute (CLSI) with the methods of serial dilution using sterile 96-well microplate readers (PLT microtiter plate ESP).[61] Ten milligrams of sample were dissolved in 1000 µL of DMSO to obtain stock solution. Hundred microliters of the Müller-Hinton Broth (Merck 110293) was loaded to the test wells. Hundred microliters of the stock solution of our material were taken and starting from the 1st well to 10th well, serial dilutions were performed and the last two wells were used as control groups. Bacterial suspensions were adjusted to 0.5 McFarland Standard and 10 microliters of bacterial suspensions, which were prepared according to the McFarland 0.5 turbidity threshold, were distributed to all samples including the control wells.[62] Orbital Shaker (PST 60HL Thermo USA) was used for 5 min in order to mix bacteria and our substance. Lid of the microplate was closed, and it was incubated at 35 °C for 18-20 h. In order to check bacterial growth, culture from each well was streaked on Müller-Hinton Agar plate using a sterile plastic loop and incubated under the same conditions. Antimicrobial activity of compounds was expressed as minimal inhibitory concentration (MIC) which is the lowest concentration of an antimicrobial agent that inhibits visible growth of the bacteria after overnight incubation. A pre-dilution of growth was determined as minimal inhibitory concentration (MIC) of that substance.[63] Antifungal activity was determined using Sabouraud Dextrose Broth and Agar (CM0147, CM0041, USA) under the same conditions.[64]

Antibacterial drugs such as gentamicin, sefazolin, amikacin, teikoplanin and meropenem, and an antifungal drug itrakonazol were used as standard control drugs for antibacterial and antifungal activity. Bacterial strains: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* MRSA ATCC 43300, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 19606. Fungal strains: *Candida albicans* ATCC 14053, *Candida glabrata* ATCC 90030.

Computational study

The ground state geometries of Ag complexes are fully optimized without symmetry constraints. The vibrational frequencies of the compounds were also calculated and all stationary points were defined and characterized as true minimums. The eigenvalues of HOMO and LUMO, global hardness (η), chemical potential (μ), electrophilicity index (ω), and dipole moments (DM) of the recent five compounds considered at DFT/B3LYP/LANL2DZ basic set via Gaussian 09, Revision E.01 program package.[65] Afterwards, *in silico* ADMET prediction was applied with help of Data Warrior 5.5.0[50] to determine their drug likeness and potential toxicity risks.

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