The palladium-based complexes bearing 1,3-dibenzylbenzimidazolium with morpholine, triphenylphosphine, and pyridine derivate ligands: synthesis, characterization, structure and enzyme inhibitions

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1. Introduction

N-heterocyclic carbene (NHC) is important supporting ligands [1] with their innovative biological effects [2]. The steric and electronic properties of NHC have contributed to their being at the forefront of many advances [3]. The NHC and phosphine ligands have played a crucial role to develop organometallic chemistry. These ligand classes, combining two-electron, neutral, and σ-donor have enriched the applications of transition metal complexes [4]. Diverse approaches have been made to add a phosphine group to the NHC skeleton in the synthesis of organometallic compounds, and metatalation has been successfully accomplished through both functionalities [5]. The halide ligand and phosphine molecule used in such complexes are thought to hydrolyze and facilitate the in vitro stabilization of complex ions formed by the metal reduction in the metal’s coordination sphere [6]. Phosphine-based ligands have widespread pharmacological applications, including antibacterial antioxidant, anticarcinogenic, antiviral, antifungal, and antitumor [7]. It has been recorded that especially phosphine-based nickel (II) and palladium (II) complexes have important bioactivities [8]. These metals-based complexes are used as valuable anticancer drugs due to their effects such as inhibiting cell division and controlling gene expression. The problem with these complexes is that they decompose into highly reactive species in solution, so do not reach their pharmacological targets such as DNA. However, this can be avoided by stabilizing the Nickel (II) and palladium (II) complexes by bulky ligands such as triphenylphosphine [6, 9].

In 2006, Organ et al reported a class of precatalysts based on Pd(II) containing pyridine derived ligands [10]. The complexes consist of one NHC, two anions (usually Cl or Br), and stabilizing throw-away pyridine derivative ligands [11, 12, 13]. Recently, new works have been reported on the antimicrobial, anticancer, antileishmanial, antitoxoplasmal, and enzyme inhibition activities of the PEPPSI type Pd(II)NHC complexes [14, 15]. In this work, pyridine, 3-chloropyridine, and 2-aminopyridine ligands were used as pyridine derivatives. Numerous in vitro studies...
have been reported in the literature regarding pyridine-based compounds as active enzyme inhibitors [16, 17, 18].

Carbonic anhydrase (CA) is a metalloenzymes group that participate in various physiologic and biochemical processes including pH control of extra/intracellular spaces by catalytic reversible hydration conversion of H$_2$O and CO$_2$ to H$^+$ and HCO$_3^-$ [19]. The CA family includes eight different and distinct species: α, β, γ, δ, ε, η, θ, and τ-CAs. Also, 16 different α-CA isozymes were defined up to now [20]. Of these CAs I-III, and VII are cytosolic forms, CAs IX, IV, XII, and XIV are membrane bounded isozymes, CA V is mitochondrial form and CA VI is secreted isoenzyme [21]. CA inhibitors (CAIs) exhibit some biological activities associated with several common diseases including cancer, glaucoma, epilepsy, obesity and osteoporosis [22]. CAs I and II isozymes are the most studied and well-known isoenzymes [23]. CA I is expressed in the gastrointestinal tract and erythrocytes. On the other hand, CA II is expressed in most of tissues including gastrointestinal tract, eye, bone osteoclasts, erythrocytes, testicle, kidney, lung and brain [24]. The reactions catalyzed by CA isoenzymes are vital in reactions and processes including ion transportation and fatty acid metabolism [25]. A high level of various CA isozymes in the body is linked to several disorders involving glaucoma, cancer, epilepsy and obesity [26, 27]. Recently, human CA Inhibitors (CAIs) have been effectively used to treat many diseases like epilepsy, intracranial hypertension, obesity and hypoxic tumors [28, 29]. CAIs were also used as important drugs for cerebral ischemia, neuro-transmission and the deterioration of cholinergic neurons are the major causes of the decline in cognitive function in AD patients [36, 37].

The acetylcholinesterase (ACHE) enzyme is an important target for Alzheimer's disease (AD) that has commonly affected the elderly people, especially after the age of sixty [32, 33]. The symptoms of AD appear gradually with age and over time. It makes the person unable to do their daily work and activities on their own [34, 35]. In the brain, the loss of neurotransmission and the deterioration of cholinergic neurons are the major causes of the decline in cognitive function in AD patients [36, 37]. One of the therapeutic approaches is AChE inhibitors (AChEIs) to limit the degradation of acetylcholine (ACh) [38]. AChEIs are able to increase the function of neural cells by increasing the ACh concentration. So, most studies have been focused on AChE inhibition and AChEIs for AD treatment [39, 40].

In the light of the above-mentioned information, the design and synthesis of more effective, new and potent CAs and AChEIs are of great importance. Recently, our research group has synthesized such complexes containing NHC ligands bearing different functional groups and determined the enzyme inhibitory effects of these complexes [12, 14, 22]. In this work, we aimed to examine the CAs and AChE inhibition activities of new (NHC)PdBr$_2$L complexes obtained by the coordination of various ligands without changing the NHC and halogen ligands coordinated to palladium metal. In this context, we synthesized (NHC)PdBr$_2$L complexes for identification of therapeutic potentials with the coordination of five different ligands (L: triphenylphosphine, morpholine, pyridine, 3-chloropyridine and 2-aminopyridine) with diverse electronic and structural properties apart from NHC and Br ligands. All synthesized complexes were characterized by NMR, FTIR methods, and elemental analysis technique. The molecular structures of three of these synthesized complexes were elucidated by the XRD method.

To our knowledge, the CAs and AChE inhibitory properties of palladium-based complexes bearing 1,3-dibenzylbenzimidazolium with morpholine, triphenylphosphine, and pyridine derivate ligands have not been reported up to date. Therefore, their CAs and AChE inhibition potentials were searched for the first time.

2. Experimental

2.1. General methods

All new complexes (2–6) containing 1,3-dibenzylbenzimidazolium ligand were synthesized by the standard Schlenk technique under an inert atmosphere. All reagents were obtained from Sigma-Aldrich, Merck, Isolab and Acros Chemical Co. The melting points were determined in glass capillaries under air with an Electrothermal-9200 apparatus. Also,
FT-IR spectra were recorded in the range of 400–4000 cm\(^{-1}\) by PerkinElmer Spectrum 100 FTIR spectrometer. Proton (\(^{1}\)H) and Carbon (\(^{13}\)C) NMR spectra were recorded by a Bruker Avance III 400 MHz NMR spectrometer operating at 100 MHz (\(^{13}\)C), 400 MHz (\(^{1}\)H) in CDCl\(_3\) with tetramethylsilane as an internal reference.

2.2. The blood sample supply

There is no clinical application in this study, so no animals or humans were used. Human erythrocytes used as carbonic anhydrase isoenzymes source in this study. Since expired waste blood was used in the study, permission was not obtained from our institution. Our institution has approved the use of expired blood samples in our experimental studies. The used human erythrocytes were the waste and expired blood that was procured from the Blood Center of Atatürk University Research Hospital. The blood donation was made by healthy volunteers on a national scale and an ethics committee certificate was not required.

2.3. Synthesis

2.3.1. Synthesis of the 1,3-dibenzylbenzimidazolium chloride, 1

This known compound 1 [41] was prepared by mixing 1-benzylbenzimidazole (1.5 g, 7.2 mmol) and benzyl chloride (0.91 g, 7.2 mmol) in acetonitrile (5 mL) at 80 °C for 24 h. Yield: 86% (2.07 g); m.p.: 233–234 °C.

2.3.2. Synthesis of the dibromo[1,3-dibenzylbenzimidazol-2-ylidene]pyridinepalladium(II), 2

This known complex 2 [42] was prepared by mixing 1,3-dibenzylbenzimidazolium chloride (200 mg, 0.6 mmol), palladium chloride (106 mg, 0.6 mmol), potassium bromide (143 mg, 1.2 mmol) and potassium carbonate (414 mg, 3 mmol) in pyridine (4 mL) at 80 °C for 16 h. Yield: 72% (278 mg); m.p.: 233–234 °C; \(\nu\) (CN): 1411 cm\(^{-1}\).

2.3.3. Synthesis of the dibromo[1,3-dibenzylbenzimidazol-2-ylidene]-3-chloropyridinepalladium(II), 3

The complex 3 was synthesized by the same method as complex 2. But, 3-chloropyridine was used instead of pyridine as solvent and ligand. Yield: 68% (276 mg); m.p.: 226–227 °C; \(\nu\) (CN): 1411 cm\(^{-1}\). Anal. Calc. for C\(_{26}\)H\(_{22}\)Br\(_2\)Cl\(_2\)N\(_3\)Pd: C: 46.05; H: 3.27; N: 6.20. Found: C: 45.96; H: 3.33; N: 6.24. \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.14 (d, 4H, \(J\) = 14.4 Hz, –NCHR\(_2\)C\(_6\)H\(_5\)); 6.98–7.54 (m, 16H, Ar–H and C\(_6\)H\(_5\)Py); 7.67 (m, 1H, C\(_6\)H\(_5\)Py); 8.88 (d, 1H, \(J\) = 5.4 Hz, C\(_6\)H\(_5\)Py); 8.98 (s, 1H, C\(_6\)H\(_5\)Py). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 53.2 and 53.8 (–NCHR\(_2\)C\(_6\)H\(_5\)); 111.5, 111.6, 123.3, 124.9, 128.0, 128.1, 128.3, 128.9, 132.6, 132.7, 134.7, 134.8, 134.9, 138.0 and 138.1 (Ar–C); 150.0, 150.6, 151.1 and 151.7 (C-Py); 163.3 (Pd–C\(_{\text{carbon}}\)).

Scheme 1. Synthesis of the (NHC)PdBr\(_2\)L complexes (2–6).
2.3.4. Synthesis of the dibromo[1,3-dibenzylbenzimidazol-2-ylidene]pyridinepalladium(II), 4

The complex 4 was prepared by mixing dibromo[1,3-dibenzylbenzimidazol-2-ylidene]pyridinepalladium(II) (129 mg, 0.2 mmol) and 2-aminopyridine (19 mg, 0.2 mmol) in chloroform (15 mL) at 25 °C for 6 h. Yield: 73% (91 mg); m.p.: 287 °C. But, morpholine (18 mg, 0.2 mmol) was used instead of 2-aminopyridine (19 mg, 0.2 mmol) in chloroform (15 mL) at 25 °C for 6 h. Yield: 70% (91 mg); m.p.: 237 °C. Anal. Calc. for C25H27Br2N3OPd: C: 46.07; H: 4.11; N: 6.32. 1H NMR (400 MHz, CDCl3); J = 128.9, 128.9, 134.7 and 134.9 (NH; ¼ 400 MHz, CDC13); δ = 5.29 (s, 2H, −NH2Py); 6.15 (s, 4H, −NCH2C6H5); 6.43 (d, 1H, J = 8.2 Hz, CHPy); 6.56 (d, 1H, J = 6.3 Hz, CHPy); 6.98–7.56 (m, 15H, Ar–H and CHPy); 8.24 (d, 1H, J = 4.8 Hz, CHPy). 13C NMR (100 MHz, CDCl3) δ: 53.6 (−NCH2C6H5); 111.3, 111.5, 114.2, 123.2, 128.1, 128.3, 128.7, 129.0, 134.8, 135.0 and 138.6 (Ar–C); 149.4 (C–Py); 158.1 (Pd–C(carbene)).

2.3.5. Synthesis of the dibromo[1,3-dibenzylbenzimidazol-2-ylidene]-triphenylphosphine palladium(II), 5

This known complex 5 [42] was prepared by mixing dibromo[1,3-dibenzylbenzimidazol-2-ylidene]pyridinepalladium(II) 2 (129 mg, 0.2 mmol) and triphenylphosphine (53 mg, 0.2 mmol) in chloroform (15 mL) at 25 °C for 6 h. Yield: 73% (91 mg); m.p.: 287–288 °C; ν(C=O) 1410 cm−1.

2.3.6. Synthesis of the dibromo[1,3-dibenzylbenzimidazol-2-ylidene]morpholinopalladium(II), 6

The complex 6 was synthesized by the same method as complex 4. But, morpholine (18 mg, 0.2 mmol) was used instead of 2-aminopyridine as ligand. Yield: 70% (91 mg); m.p.: 237–238 °C; ν(C=O) 1410 cm−1; ν(NH) 3305 cm−1. Anal. Calc. for C26H24Br2N4Pd: C: 46.74; H: 4.33. 1H NMR (400 MHz, CDCl3); δ 2.73 (s, 1H, −NH(CH2CH2)2O); 2.90 (t, 2H, J = 8.9 Hz, −NH(CH2CH2)2O); 3.42 (m, 4H, −NH(CH2CH2)2O); 3.74 (d, 2H, J = 8.5 Hz, −NH(CH2CH2)2O); 6.00 (s, 4H, −NCH2C6H5); 6.96–7.48 (m, 14H, Ar–H). 13C NMR (100 MHz, CDCl3) δ: 48.3 (−NH(CH2CH2)2O); 53.6 (−NCH2C6H5); 68.1 (−NH(CH2CH2)2O); 111.5, 123.2, 128.0, 128.3, 128.9, 134.7 and 134.9 (Ar–C); 149.4 (C–Py); 165.9 (Pd–C(carbene)).

2.4. Crystallography

Single crystal x-ray diffraction data of the complexes 3, 4, and 6 were obtained from a Rigaku Oxford XCalibur diffractometer including EOS CCD detector using MoKα radiation with the operation condition of 50 kV and 40 mA (λ = 0.7107 Å) at 25 °C. CrysAlis software package was carried out for the data collections, cell refinements, data reductions and absorption corrections [43]. To perform structure solution and refinement process for each complex, the methods of SHELXT [44] and SHELXL [45] were applied, respectively via OLEX2 software [46]. All of the non-hydrogen atoms were processed as an anisotropic thermal ellipsoid. The riding model was used for determination of hydrogen atoms’ positions. Crystallographic data of the all complexes were demonstrated in Table 1.

2.5. Biochemical studies

2.5.1. hCA isoenzymes activity assay

This study, both hCA isoenzymes were obtained from expired waste blood human red blood cells using Sepharose-4B-L-Tyrosine-sulfanilamide affinity (SB LTS) chromatography [47] as described previously [48], which was used as an affinity matrix for selective purification of hCA isoenzymes [49]. Our institution has approved the use of expired blood samples in our experimental studies. Both hCA isoenzymes activity was spectrophotometrically recorded according to Verpoorte et al. [50] and detailed in prior studies [51]. p-Nitrophenylacetate (PNA) was used as substrate for enzymatic reaction [52]. One CA isoenzyme unit is defined as the CA amount that had absorbance change at 348 nm of PNA to p-nitrophenolat ions (PNP) over a period of 3 min at 25 °C [53]. The quantity of protein during the purification procedure was determined according to prior study [54]. Bovine serum albumin was used as a standard protein [55, 56]. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for screening of purity of both isoenzymes [57].

2.5.2. Acetylcholinesterase (AChE) activity assay

Acetylcholinesterase (from Electrophorus electricus) was obtained from Sigma-Aldrich. The effects of benzimidazolium salt 1, palladium-based
complexes (2–6) and reference compounds including tacrine and donepezil on AChE activity were performed according to Ellman’s method [58] as given previously [59]. The hydrolysis of acetylcholine iodide (AChI) by AChE was recorded at 412. Briefly, an aliquot (0.1 mL) of AChE enzyme solution (5.32 μL of 2.5 EU) and reference compounds including tacrine and donepezil was incubated for 10 min at 25 °C, and the hydrolysis of acetylcholine iodide (AChI) was monitored spectrophotometrically by the formation of the yellow thiocholine, which released by enzymatic hydrolysis of AChI with AChE was recorded at 412. Brie.

### 2.5.3. Inhibition kinetic studies

To investigate the in vitro inhibition mechanism of the benzimidazolium salt 1 and palladium-based complexes (2–6), kinetic studies were realized with the different concentrations of benzimidazolium salt 1, palladium-based complexes (2–6), and substrate. IC₅₀ values and dawning of Lineweaver-Burk curves [61] were calculated as given previously [62, 63]. From the observed data, IC₅₀ and Kᵢ values for benzimidazolium salt 1 and palladium-based complexes (2–6) were computed, and the types of inhibition of both isoenzymes were evaluated as in previous study [64]. The lower IC₅₀ shows the higher inhibition effects and the easier it is to use. The IC₅₀ value is the most practical way for evaluation of inhibition affinities [65].

### 3. Results and discussion

#### 3.1. Synthesis

The synthetic route for Pd(II)NHC complexes containing 1,3-dibenzyldiazolidazolium ligands 2–5 has been defined. The PEPPSI type Pd(II)NHC complexes 2 and 3 were prepared by mixing 1,3-dibenzyldiazolidazolium chloride [41], palladium chloride (PdCl₂), potassium bromide (KBr) and potassium carbonate (K₂CO₃) in pyridine/3-chloropyridine (4 mL) at 80 °C and incubated for 10 min at 25 °C. Then, 50 μL of AChE enzyme solution (5.32 μL of 2.5 EU) was added. The reaction was allowed to be initiated upon addition of 50 μL of AChI. The hydrolysis of AChI was monitored spectrophotometrically by formation of the yellow 5-thio-2-nitrobenzoic anion, as a result of the reaction of DTNB with thiocholine, which released by enzymatic hydrolysis of AChI with maximum absorption at 412 nm [60].

#### 3.2. NMR study

The structures of all complexes were fully characterized by using ¹H NMR, ¹³C NMR and, FTIR spectroscopic methods and elemental analysis
technique. All data of these spectra were fully consistent with the proposed formula. For the $^1$H NMR spectra, the data given below were observed. The acidic proton peak of the starting material (1,3-dibenzylbenzimidazolium chloride) [41] observed around 12.5 ppm was not observed in the spectra of the new PEPPSI type Pd(II)NHC complexes 2 and 3. Furthermore, characteristic proton peaks of the pyridine/3-chloropyridine ligand were showed between 8.88 and 9.00 ppm. However, the pyridine proton peaks that it observed between 8.88 and 9.00 ppm in the starting complex (2) were not observed in the $^1$H NMR spectra of the other complexes (4–6). The amino (NH$_2$) protons in the 2-aminopyridine group for complex 4 were observed at 5.29 ppm as singlets. The amino (NH) proton in the morpholine group for complex 6 was observed at 2.73 ppm as singlets. Also, the CH$_2$ protons in the morpholine group for complex 6 were observed at 2.90, 3.42, and 3.74 ppm as triplet, multiplet, and doublet, respectively [18]. The significant increase in the number of aromatic protons between 7.00 and 8.00 ppm proves the presence of the triphenylphosphine group in the structure of complex 5. Benzyllic CH$_2$ protons were observed as singles around 6.00 ppm for complexes 2–4, complex 6, and as doublet at 4.78 ppm for complex 5. For the $^{13}$C NMR spectra, the data mentioned below were observed. The carbon peak of the starting material (1,3-dibenzylbenzimidazolium chloride) [41] observed around 143 ppm was not observed in the $^{13}$C NMR spectra of the new PEPPSI type Pd(II)NHC complexes 2 and 3. Furthermore, characteristic carbon peaks of the pyridine/3-chloropyridine ligand were observed between 150 and 152 ppm. However, the pyridine carbon peaks that it observed between 150 and 152 ppm in the starting complex (2) were not observed in the $^{13}$C NMR spectra of the other complexes (4–6). The significant increase in the number of aromatic carbons between 123 and 135 ppm proves the presence of the triphenylphosphine group in the structure of complex 5. Benzyllic CH$_2$ carbons were observed at around 48.3 ppm for complex 6. The carbons in the morpholine group for complex 6 were observed at 48.3 (C–N) and 68.1 (C–O) ppm [18]. The characteristic carbene peaks (2-C$_{carbene}$) were observed 163.2, 163.3, 158.1, 165.9, and
176.7 ppm for all complexes 2–6, respectively. Finally, the presence of a phosphorus peak at 26 ppm in the $^{31}$P NMR spectrum for complex 5 proved that the triphenylphosphine ligand is coordinated to the palladium metal.

### 3.3. FTIR study

The FTIR spectra, the data given below were observed. The C–N stretching frequency observed at 1557 cm$^{-1}$ for starting material (1,3-dibenzylbenzimidazolium chloride) [41] were observed around 1410 cm$^{-1}$ for all complexes 2–6. The N–H (primer) stretching frequency for the amino group in complex 4 was observed at 3325 and 3448 cm$^{-1}$. The N–H (seconder) stretching frequency for the morpholine group in complex 6 was observed at 3305 cm$^{-1}$. The C–O stretching frequency for the morpholine group in complex 6 was observed at 1027 cm$^{-1}$. The P–C$_{Ar}$ stretching frequency for the triphenylphosphine group in complex 5 was observed at 1094 cm$^{-1}$. Also, it was shown that the calculated and experimentally determined elemental analysis were found to be very close to each other.

### 3.4. Structural details of the complexes

Heteroatom and functional group incorporation to the molecule backbone is highly effective in tuning the molecular structures, non-covalent interactions including non-classical hydrogen bonds, π–π stacking interactions, C–H···π, halogen ···π, and short interactions and their packing arrangements. Therefore, in the light of the data obtained from single crystal X-ray crystallography, the noticeable discrepancy was seen in the 3D molecular solid-state structures of the complexes and their packing arrangements created by the noncovalent interactions due to this reason. The molecular structures of the complexes were given in Figure 1. The crystallographic details including the bond lengths and angles of the complexes which are in good agreement with the similar structures reported in the literature were listed in Table 2 [66]. The crystallographic results revealed that complex 3 crystallizes in the triclinic space group P-1 with the two molecules in the asymmetric unit while the complex 6 and complex 4 crystallize in the monoclinic crystal system with space group $\text{I2}/a$ and $\text{P2}_1/\text{n}$, respectively.

The 3D molecular structure of the trans-Pd (II) complexes (3, 4, and 6) demonstrated a slightly distorted square planar geometry with the palladium (II) center coordinated through the nitrogen (N1) atom which is the morpholine nitrogen atom and the pyridine nitrogen atom for the molecules 3 and 4 and a carbene carbon atom (C1) of the NHC ligand via two bromine atoms (Br1 and Br2). The bond angles around the Pd atoms (N1–Pd–Br1: 92.4(3), 90.18(18), 90.79(10); N1–Pd1–Br2: 89.93(3)$^\circ$, 92.68(8)$^\circ$, 90.02(10)$^\circ$; C1–Pd1–Br1:89.2(4)$^\circ$, 88.65(9)$^\circ$, 88.46(13)$^\circ$; C1–Pd1–Br2: 88.7(4)$^\circ$, 88.93(4)$^\circ$, 88.60(13)$^\circ$ for all complexes) are very close to 90$^\circ$ due to the distorted square planar structures of coordination of the platinum (II) metal as reported for similar N-heterocyclic carbene complexes [67, 68] (Table 2). The coordination planes PdBr$_2$CN of the complex 3, 4, and 6 are essentially planar to the molecular plane, with an overall r.m.s. deviation of 0.017 Å, 0.057 Å, and 0.034 Å, respectively.

The dihedral angles between the NHC and [PdBr$_2$CN] mean planes are 109.98 (2)$^\circ$, 92.259$^\circ$ and 94.824 (9)$^\circ$ for 3, 4, and 6, respectively, while those between the pyridine and [PdBr$_2$CN] mean planes are 142.12$^\circ$ and 105.67 (1)$^\circ$ for 3 and 4, respectively and between the morpholine and [PdBr$_2$CN] mean planes is 106.769 (2)$^\circ$ for 6. This significant difference is likely due to van der Waals packing contacts raised from different functional groups with the ligands of neighboring complex molecules. The Pd-Carbene bond lengths of the complexes are an average of 1.962 Å are slightly shorter than the sum of the covalent radii of the associated atoms (dcov: Pd–C = 2.07 Å) since it has greater σ donating ability than that of usual NHC complexes [69]. The Pd – Br bond lengths which have an average of 2.428 Å are between the expected values compared to the N-heterocyclic carbene palladium complexes. Additionally, Pd–N bond lengths (2.116(10) Å, 2.096(4) Å, and 2.136 (3) Å, for 3, 4, and 6 respectively) are consistent with reported similar trans-Pd (II) complexes, it is longer than the sum of individual covalent radii of Pd and N (1.983 Å), which is due to a strong trans effect of the NHC ligand residing diagonally opposite to the pyridine and morpholine rings [69, 70]. The morpholine ring of the complex 6 has a near chair conformation with puckering parameters q2: 0.554 (5), theta: 172.3 (4)$^\circ$, and phi:174.4 (4)$^\circ$ parameters.

The crystal packing of the Pd-based NHC complexes is determined by noncovalent intermolecular interactions, which also have significant effects on their biological activity results. Therefore, to further understand the biological activity of the studied complexes, their non-covalent interactions and packing structures in solid-phase were examined from the single-crystal X-ray crystallography data. The molecules 3 and 4 have chloropyridine and aminopyridine ligands, respectively that they can affect the geometry of the complex (bond lengths, angles, isomerism of the complex molecule). Interplay between intramolecular hydrogen bonding and the molecular geometry of the complex molecule has attracted great attention to obtain favorability of binding of ligands onto coordinatively unsaturated metal centers. Since a ligand’s ability to form hydrogen bonds with other ligands can have an important effect on coordination geometries. Incorporation of neutral ligands to the complex molecule such as pyridine and amino pyridine which have excellent neutral Lewis bases coordinating to metal ions creates additional hydrogen bonding in the primary coordination sphere, therefore they have used in several examples of coordination chemistry [71,72].
Aminopyridine ligand of the molecule 4 creates strong intermolecular hydrogen bond to the bromine atoms of the chelate plane and C20 carbon atom bounded to imidazole ring (N4–H4B⋯Br2; C20–H20A⋯N4) with change of the molecular packing of the complex. In addition, the presence of the weak intramolecular hydrogen bonding (C8–H8⋯Br2) in the molecule 3 result in a lengthening of the Pd1–N1 (Pd1–N1: 2.116 Å for molecule 3 and 2.096 Å for molecule 4) distance, as the intermolecular hydrogen bond draws the pyridine molecule away from the metal atom. When the a NH2 substituent is present on the pyridine ring, the configuration of the molecule changes which allows the aminopyridine ligands to penetrate deeper into the coordination sphere as can be seen by the significant shortening of the Pd1–N1 (2.096 Å) bond distance.

The dihedral angle between the phenyl and chelate rings (pyridine and [PdBr2CN] mean planes are 142.123° and 105.67 (1)° for 3 and 4, respectively and between the morpholine and [PdBr2CN] mean plane is 106.769 (2)° for 6) and planarization of the complexes were affected by the type of the ligands. Morpholine ligand increase the planarity of the molecules which changes their molecular arrangements in solid phase.

According to crystallographic results, the molecular arrangements of the complex 3 is dominated by the nonclassical hydrogen bonds (C8–H8⋯Br2 and C25–H25⋯Br3) and short interactions (Br1–H36 (2.99 Å) intermolecular short interactions and Br3–H46 (2.69 Å) intramolecular short interactions) while the hydrogen bonds, π⋯π stacking interactions, C–H⋯π, halogen⋯π interactions are responsible for the molecular arrangements of the complex 4 (Table 3). The molecular packing arrangement of complex 3 is given in Figure 2.

Due to the strong intermolecular interactions of the molecule 4 allow the formation of strong π⋯π stacking interactions (Cg5⋯Cg2: 4.895 Å; Cg2:N1–C8/C12; Cg5:C21/C26) and dimeric structure since adjacent molecules in solid phase approaches each other thanks to electrostatic interactions in crystal phase. Beside the π⋯π stacking interactions, unlike the molecule 3, C–H⋯π and Pd⋯Br⋯π interactions were observed in crystal structure of the molecule 4. These interactions enhance the rigidity and stability of the molecule 4 in its molecular arrangements.

Introducing an morpholine ring to the complex leads to formation of intramolecular hydrogen bonds (N1–H1⋯Br1; C22–H22A⋯Br2) which cause to distortion of the coordination geometry and extension of the Pd1–N1 bonds (2.136 (3) Å), as compared to the other molecules. Similar shortening of the Pd–N bond length when the morpholine ring is present have been observed the previous studies. The presence of the intramolecular H-bonding in the crystal structure of the complex which are molecules 3 and 6 enable the extension of the Pd1–N1 bonds since the H atoms attracts the ligands from the Pd metal ion. This situation is similar to previous studies given in the literature [73, 74]. In addition, due to the presence of the electron acceptor O atom which influence electron distribution in the molecule, molecule 6 has strong intermolecular hydrogen bonding (C8–H8A⋯O1) for generation of the C–H⋯π (C23–H23A⋯C3; C3: C2/C7) and intermolecular π⋯π stacking interactions (Cg4⋯Cg4: 4.735 Å; Cg4:C9/C14).

The complexes 4 and 6 displayed supramolecular dimer by their intermolecular interactions (C20⋯H20A⋯N4 and N4⋯H4B⋯Br2 hydrogen bonds for complex 4, Br1⋯H15A short interactions between the neighboring molecules) leading to centre-symmetric R₂(15) and R₂(12) graphset motifs, respectively (Figure 3). These dimeric structures of the complexes enhance their structural rigidity in the solid phases of the molecules 4 and 6.

The packing in the crystal structures of the complexes 4 and 6 is achieved by the intermolecular π⋯π stacking interactions (Cg4⋯Cg4: 4.735 Å for complex 6 and Cg5⋯Cg2: 4.895 Å for the complex 4; Cg2:N1–C8/C12; Cg4:C9/C14; Cg5:C21/C26), nonclassical intra and intermolecular hydrogen bonds (N4–H4B⋯Br2 and C20⋯H20A⋯N4 hydrogen bonds for complex 4 and C8⋯H8⋯O1 and C22–H22A⋯Br2 for complex 6) along the (010) plane (Table 3; Figure 4).

3.5. Enzyme inhibition results

Recently, many compounds associated with enzyme inhibition have been evaluated to improve human health and demonstrate the health importance of inhibitors [75, 76, 77]. The synthesized benzimidazolium salt 1 and a series of novel the Palladium-based complexes bearing 1, 3-dibenzylimidazolium with morpholine, triphenylphosphine, and pyridine derivate ligands, (2–6), were tested towards cytosolic isofoms hCA I, hCA II and AChE.

1. All the novel synthesized complexes effectively inhibited cytosolic hCA I isoenzyme with IC₅₀s ranging from 31.5 to 115.5 nM. IC₅₀ value of an inhibitor is the required concentration to inhibit half of the
maximum enzyme activity [78]. Also, Ki values varies in ranging of 10.0 ± 1.4–68.5 ± 11.5 nM. All the studied compounds exhibited the best inhibition effects when compared to AZA (Ki: 52.1 ± 4.2 nM). Among them, the complex of dibromo 1,3-dibenzylbenzimidazol-2-ylidene-3-chloropyridinepalladium (II) (3) demonstrated the best inhibition (Ki: 10.0 ± 1.4 nM). The inhibition effects of benzimidazolium salt 1 and palladium-based complexes bearing 1,3-dibenzylbenzimidazolium with morpholine, triphenylphosphine, and pyridine derivate ligands (2–6) on hCA I were decreased as follows: 3 (Ki: 10.0 ± 1.4 nM) > 6 (Ki: 16.9 ± 0.3 nM) > 4 (Ki: 18.1 ± 3.0 nM) > 2 (Ki: 28.4 ± 1.2 nM) > 5 (Ki: 40.3 ± 2.0 nM) > 1 (Ki: 68.5 ± 11.5 nM) (Table 4). According to results, chlorine binding to the 3rd position of the benzene ring of complex 2 increased the 2.83-fold inhibition, binding amino group 2nd position of the benzene ring increased the 2.83 times inhibition.

4. Conclusions

As a result, four new Pd(II)NHC complexes were reported in this work. All complexes’ structures were fully characterized by using spectroscopic methods (1H NMR, 13C NMR and FTIR) and elemental analysis technique. The existence of Pd-carbene peaks in the expected region in 13C NMR shows that the structure was consistent with the proposed formula. A single peak observed in the 31P NMR spectrum proves that PPh3 coordinates to the Pd center. The C–N stretching vibrations in the FTIR spectrum prove that the NHC ligand is coordinated to the Pd center. N–H stretches of primary and secondary amines demonstrate that the ligand of 2-aminopyridine and morpholine are coordinated to the Pd center, respectively. The crystal structures of three complexes were determined. According to crystallographic results, incorporation to aminopyridine ligand leads to generation of intermolecular hydrogen bonding which result in different packing arrangements, molecular geometry and stability in the solid phase of the complex molecule. Mor- pholine ring in the complex enable to formation of intramolecular hydrogen bonds, which cause to distortion of the coordination geometry and extension of the Pd1–N1 bonds, as compared to the other molecules.

As a brief, the presence of the intramolecular H-bonding in the crystal structure of the complex result in the extension of the Pd1–N1 bonds. Due to change in the molecular conformation with the different ligands form differences in the molecular arrangements and number and type of the inter-intermolecular interactions therefore it allows to increase stability and rigidity of the crystal structure in solid phase. The enzyme inhibition activities of benzimidazolium salt 1 and palladium-based complexes bearing 1,3-dibenzylbenzimidazolium with morpholine, triphenylphosphine, and pyridine derivate ligands (2–6) were examined against AChE and hCAs I and II isofoms, which associated with some common and global diseases including AD, epilepsy and glaucoma.

Declarations

Author contribution statement

Aydın Aktas: Performed the experiments; Wrote the paper.
Gül Yakal, Yeliz Demir: Performed the experiments.
İlhami Gölünç: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Muhittin Aygün: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Yetkin Gök: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data associated with this study has been deposited at Crystallographic data as .cif files for the structures reported in this paper have been
Additional information

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