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Araştırma Makalesi / Research Article

Synthesis and Characterization of Novel Benzimidazolium Type NHC Molecule and Its Silver Complex and Their Theoretical Analysis for Potential Anti-Cancer Activity

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Abstract

N-heterocyclic carbenes have initially attracted great attention of the chemists in consequence of their catalytic activity. The favorable results were also obtained in bioactivity studies for N-heterocyclic carbenes, and their transition metal complexes in following years. Theoretical methods deliver beneficial data about the several activities of molecules. One of them is the frontier orbital analysis, which supplies data about the reactivity of molecules. Another method is investigation of the molecular activity against crystals of certain macromolecules using molecular docking methods. In this study, novel 1-allyl-3-(cyclohexylmethyl)benzimidazoliumbromide and bromo[1-allyl-3-(cyclohexylmethyl)benzimidazolium-2-ylidene]silver(I) were synthesized and characterized. The characterizations were also supported by spectroscopical and theoretical methods. In order to have foresight about the bioactivities of these molecules, molecular docking methods were used to get information about the interactions between molecules and VEGFR-2, which regulates the angiogenesis, and DNA.

Keywords: N-Heterocyclic Carbenes, VGEFR-2, Silver Molecules, Molecular Docking, DFT

Benzimidazolyum Tipi Yeni NHC Molekülü ve Gümüş Kompleksinin Sentezi ve Karakterizasyonu ve Potansiyel Antikanser Aktivite için Teorik Analizi

Öz

N-heterosiklik karbenler başlangıçtan günümüzü katalitik aktiviteleri dolayısıyla kimyagerlerin ilgisini çekmektedir. Sonraki yıllarda N-heterosiklik karbenlerin ve bunların metal komplekslerinin biyoaktivite çalışmalarından olumlu sonuçlar alınmıştır. Teorik metotlar moleküllerin çeşitli aktiviteleri ile ilgili kullanışlı bilgiler sunarlar. Bu teorik yöntemlerde biri moleküllerin reaktiviteleri hakkında bilgiler sunan sınır orbital analizidir. Bir başka metot ise moleküler doking yöntemleri kullanılarak belirli makromoleküllerin kristallerine karşı moleküler aktivitenin incelenmesidir. Bu çalışmada, yeni 1-allil-3-(siklohekzillmetil)benzimidazolyumbromid ve bromo[1-allil-3-(siklohekzillmetil)benzimidazolyum-2-yliden]gümüş(I) molekülleri sentezlenerek karakterize edilmiştir. Moleküllerin karakterizasyonları ayrıca spektroskopik ve teorik yöntemlerle desteklenmiştir. Bu moleküllerin biyoaktiviteleri ile ilgili fikir sahibi olmak için, anjiyogenezi düzenleyen VEGFR-2 ve DNA ile etkileşimleri moleküler doking yöntemleri ile analiz edilmiştir.

Anahtar Kelimeler: N-Heterosiklik Karbenler, VEGFR-2, Gümüş Molekülleri, Moleküler Doking, DFT

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1. Introduction

N-heterocyclic carbenes (NHCs) have been studied for their many properties since they were firstly synthesized (Hopkinson et al, 2014; Hermann and Köcher, 1997). Well-known catalyst NHCs can be easily achieved, and refashioned (Hermann, 2002; Diez-Gonzalez et al, 2009). Additionally, the bioactivities of many NHC-metal complexes have been frequently studied (Şahin et al, 2022; Fontes et al, 2022; Aber et al, 2014). Especially, ruthenium- and rhodium-NHC complexes' antibacterial activities are well known (Ott, 2017; Hamd et al, 2021). After advances in metal-based anticancer drugs, metal-NHC complexes have also been researched for their anticancer activity. Anti-cancer activities that were acquired from Au-NHC complexes are noteworthy (Karaaslan et al, 2020). Moreover, Ag-NHC complexes, generally known as anti-infective, have been also progressed for anticancer properties (Şahin-Bölükbaşı, and Şahin, 2019).

Angiogenesis which occurs in cell growth and wound healing processes is the formation of new blood vessels from a pre-existing vasculature (Folkman, 1984). However, excessive angiogenesis could induce pathologies such as metastasis, increment of existed tumor and new tumor formation (Nishida et al, 2006; Carmeliet and Jain, 2000). In this case, control the angiogenesis systems may be a promising way for cancer therapy. Vascular endothelial cells are regulated by vascular endothelial growth factor (VEGF) (Ferrara, 2009; Hoeben et al, 2004). The activity of VEGF is administrated by vascular endothelial growth factor receptors (VEGFR) (Olofsson et al, 1996; Ferrara, 2004). VEGFR-1 and VEGFR-2 which are the sub-types of VEGFR have different kinase activation potentials. VEGFR-2 triggers signaling pathways and plays more important role on angiogenesis than VEGFR-1 (Rahimi, 2006; Dias et al, 2000). Thus, the activation of VEGFR-2 could manage some pathologies of many diseases.

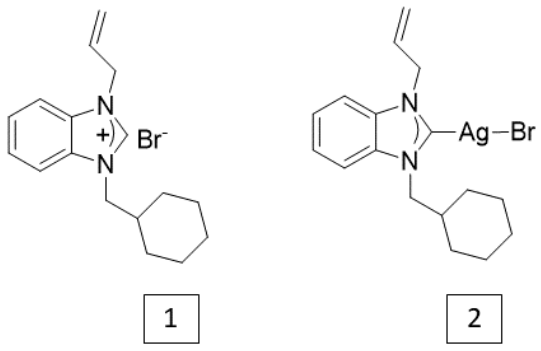


Figure 1. Molecular structures of **1** and **2**

The molecular docking methods are approved as an important tool for examining the interactions of new molecules against biological macromolecules such as enzymes, proteins, DNA

(Torres et al, 2019; Trşpathi and Misrz, 2017). It is even possible to have some valuable data about the properties of unsynthesized molecules by this method. In this study, interactions of novel 1-allyl-3-(cyclohexylmethyl)benzimidazoliumbromide (1) and bromo[1-allyl-3-(cyclohexylmethyl)benzimidazolium-2-ylidene]silver(I) (2) which were synthesized (Figure 1) and characterized for this research (Fig. 1) against VEGFR-2 and DNA were investigated with molecular docking methods.

2. Materials and Methods

Synthesis of 1-allyl-3-(cyclohexylmethyl)benzimidazoliumbromide, 1

Benzimidazole (10 mmol) was appended to NaH (11 mmol) solution in tetrahydrofuran (20 mL) in a schlenk tube and the solution was mixed for 1 h at room temperature (RT). Then, allyl bromide (10.1 mmol) was also appended to the mixture and the solution was stirred for 24 h at 60 °C. The solution was cooled to RT and tetrahydrofuran was evaporated under light pressure. The resulting solid were resolved in dichloromethane (50 mL). 1-allylbenzimidazole was achieved by the distillation of the last solution. 1-Allylbenzimidazole (1 mmol) and cyclohexylmethyl bromide (1 mmol) were mixed in dimethyl formamide (5 mL) for 24 h at 80 °C. At the end of this period, 1-allyl-3-(cyclohexylmethyl)benzimidazoliumbromide (**1**) was precipitated. Compound **1** was filtered and washed with diethyl ether. Yield: 78%. m.p. 90-91 °C. FT-IR $\nu_{(CN)}$: 1557 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.19-1.25 (m, 5H, $CH_2C_6H_{11}$), 1.65-1.75 (m, 5H, $CH_2C_6H_{11}$), 2.04-2.06 (m, 1H, $CH_2C_6H_{11}$), 4.48 (d, 2H, $CH_2C_6H_{11}$, *J*= 8 Hz), 5.37 (d, 2H, NCH_2CHCH_2, *J*= 4 Hz), 5.46-5.52 (m, 2H, NCH_2CHCH_2), 6.14 (quint, 1H, NCH_2CHCH_2, *J*= 4 Hz), 7.63-7.69 (m, 2H, Ar-*H*), 7.73-7.77 (m, 2H, Ar-*H*), 11.37 (s, 1H, NC*H*N). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ (ppm): 25.3, 25.7, 30.4, 37.9 ($CH_2C_6H_{11}$), 50.1 ($CH_2C_6H_{11}$), 53.4 (NCH_2CHCH_2), 121.7 (NCH_2CHCH_2), 129.7 (NCH_2CHCH_2), 113.3, 113.7, 127.2, 131.3, 131.8 (Ar-*C*), 143.0 (N*C*HN).

Synthesis of bromo[1-allyl-3-(cyclohexylmethyl)benzimidazolium-2-ylidene]silver(I), 2

The dichloromethane solution of 1-allyl-3-(cyclohexylmethyl)benzimidazoliumbromide (1 mmol) and silver (I) oxide (0.8 mmol) were blended at RT for 24 hours under dark. The last solution was concentrated after the filtration on celite. The diethyl ether was added to the latter solution and the solid white product (**2**) precipitated and washed with diethyl ether after the isolation by filtration. Yield: 74%. m.p. 106-107 °C. FT-IR $\nu_{(CN)}$: 1394 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.15-1.22 (m, 5H, $CH_2C_6H_{11}$), 1.65-1.75 (m, 5H, $CH_2C_6H_{11}$), 1.96-1.98 (m, 1H, $CH_2C_6H_{11}$), 4.25 (d, 2H, $CH_2C_6H_{11}$, *J* = 8 Hz), 5.05 (d, 2H, NCH_2CHCH_2, *J*= 8 Hz), 5.22 (d, 1H, NCH_2CHCH_2, *J*= 16 Hz),

5.32 (d, 1H, NCH_2CHCH_2, *J*= 12 Hz), 6.01 (quint, 1H, NCH_2CHCH_2, *J*= 4 Hz), 7.38-7.50 (m, 4H, Ar-*H*). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ (ppm): 25.6, 26.0, 31.1, 38.4 ($CH_2C_6H_{11}$), 51.9 ($CH_2C_6H_{11}$), 55.8 (NCH_2CHCH_2), 119.2 (NCH_2CHCH_2), 125.1 (NCH_2CHCH_2), 111.8, 111.9, 131.8, 133.7, 134.2 (Ar-*C*), no peak ($C_{carbene}$-Ag).

Theoretical Calculation Methods

Molecules are optimized by using the BP86 functional with a def2-SVP def2-SVP/J basis set, KDIIS SOSCF and the tightscf options with ORCA version 4.1 (Becke, 1988; Perdew, 1986; Neese, 2012; Neese et al, 2020; Neese, 2022). AutoDock 4.2 were used for molecular dockings performance against both DNA dodecamer (PDB id:1bna) and VEGFR-2 (PDB id: 1ywn) which are acquired from RCSB web site (Trott and Olson, 2010; Miyazaki et al, 2005; Drew et al, 1981). Waters in the target crystals were removed, only polar hydrogen atoms were considered in processes and Kollman charges were appreciated. Molecular docking performances were started at a randomized position and Gasteiger charges used for ligand molecules (Üstün and Şahin, 2022; Wei et al, 2002). According to Koopmans Theorem, global chemical reactivity descriptors were calculated by following equations (Koopmans, 1934; Choudhary et al, 2019; Rajkumar et al, 2020):

$$IP = -E_{HOMO} \quad (1)$$

$$EA = -E_{LUMO} \quad (2)$$

$$\chi = -\mu = -\frac{I+A}{2} \quad (3)$$

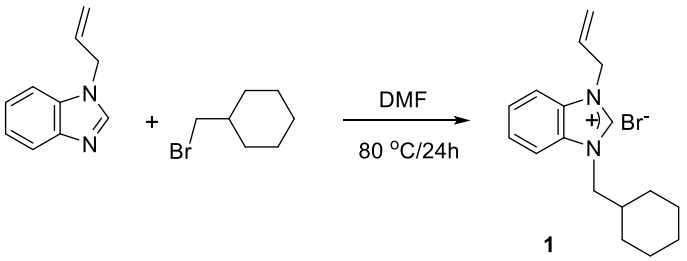
$$\eta = \frac{I-A}{2} \quad (4)$$

$$S = \frac{1}{2\eta} \quad (5)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (6)$$

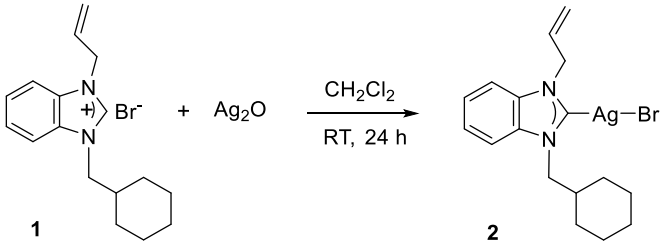
3. Findings and Discussion

The N-heterocyclic carbene precursor (**1)** and its Ag(I)-NHC complex (**2**) were freshly achieved according to the previous published studies (Şahin-Bölükbaşı, and Şahin, 2019). 1-Allylbenzimidazole was reacted with cyclohexylmethyl bromide at 80 ^oC in dimethyl formamide. N-heterocyclic carbene precursor (**1**) was obtained (Scheme 1).



Scheme 1. Synthesis of compound **1**

N-heterocyclic carbene precursor (**1**) reacted with Ag_2O in dichloromethane for a day and Ag(I)-NHC complex (**2**) was yielded as shown in Scheme 2.



Scheme 2. Synthesis of compound **2**

Both of the molecules have good solubility and are soluble in most of polar organic solvents. The ligand and the silver complex are highly stable both in air and moisture but, it must be noted that Ag(I)-NHC complex is not stable under light. The structure of the compounds was successfully clarified by ^{1}H and $^{13}C\{^{1}H\}$ NMR spectroscopies.

In the ^{1}H NMR spectra, NC*H*N acidic proton of **1** were detected at 11.37 ppm. The formation of the N-heterocyclic carbene ligand was confirmed by this characteristic pick whilst the formation of its Ag(I)-NHC complex was also confirmed by the disappearance this peak and the peak was not seen at complex spectra as expected (Figure 2). In $^{13}C\{^{1}H\}$ NMR spectra of the **1**, N*C*HN carbon to which the acidic proton is bounded was seen at 143.0 ppm. However, in $^{13}C\{^{1}H\}$ NMR spectra of **2**, this peak has not appeared and disappearance of the signal the carbon proved formation of Ag(I)-NHC complex. After complexation, N*C*N carben resonance on the **2** should shift much downfield region compared to the **1**. But this peak could not be seen in the $^{13}C\{^{1}H\}$ NMR spectra (Figure 2).

The FT-IR spectra of the compounds contain some characteristic bands of the stretching vibrations of the C=N, C-N, C-H and C=C groups. Benzimidazole ring C=N vibrations of NHC salt was assigned at 1557 cm^{-1}. This vibration was seen with a shift in the Ag(I)-NHC complex at 1394 cm^{-1}. This negative shift is because of the electropositive metal center which pulls electron density towards itself and as a result of C=N vibrations shifts to the lesser energy region in the complex. And also, this shifting is another evidence of formation Ag(I)-NHC complex.

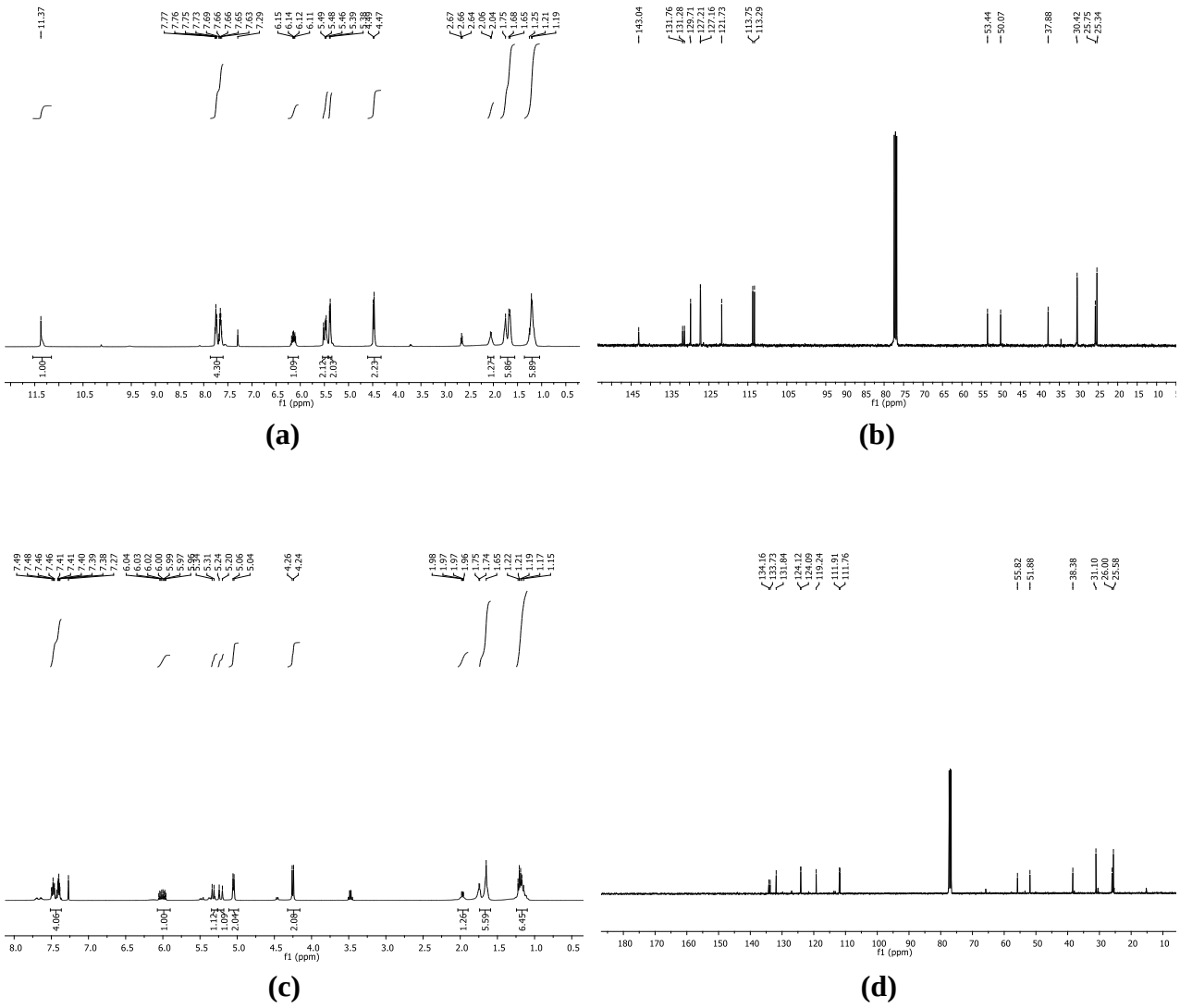


Figure 2. ^{1}H (a and c) and $^{13}C\{^{1}H\}$ (b and d) NMR spectra of **1** and **2**

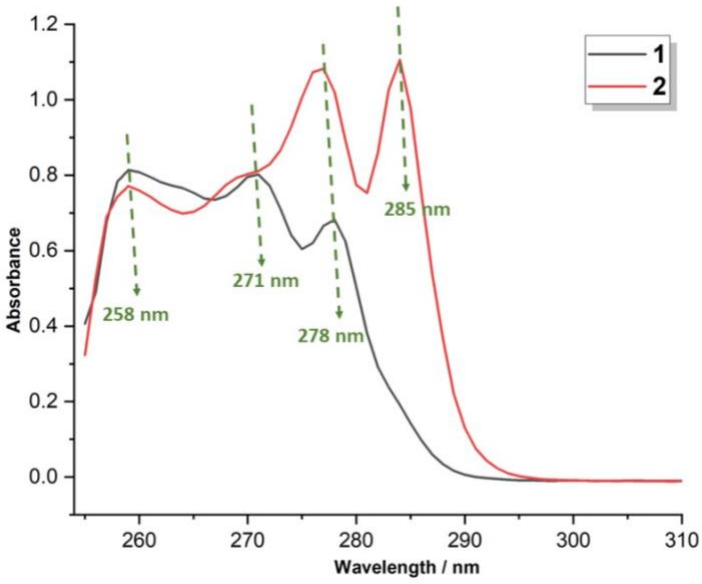


Figure 3. UV-Vis. absorption spectra of **1** and **2**

In addition to the characterization, spectroscopic analysis of both molecules were performed. Both the ligand and the complex gave a broad band at 258 nm, which can be considered a shoulder. While the band detected at 271 nm was a shoulder for the complex, it was recorded as a distinct band for the ligand. At 278 nm, evident bands were recorded for both the ligand and the complex. In the complex molecule, unlike the ligand molecule, a clear band was recorded at 285 nm, which can be considered as a MLCT (Üstün et al, 2016) (Figure 3). The extinction coefficients of these bands were recorded by preparing three sets of solutions of each molecule at five different concentrations. The extinction coefficients calculated for the ligand at 258, 271, and 278 nm are 6236.5, 6520.0, and 5515.7 $M^{-1}cm^{-1}$, respectively. For the complex, extinction coefficients for 258, 271, and 285 nm were determined as 7638.9, 9495.4, and 9107.8 $M^{-1}cm^{-1}$, respectively.

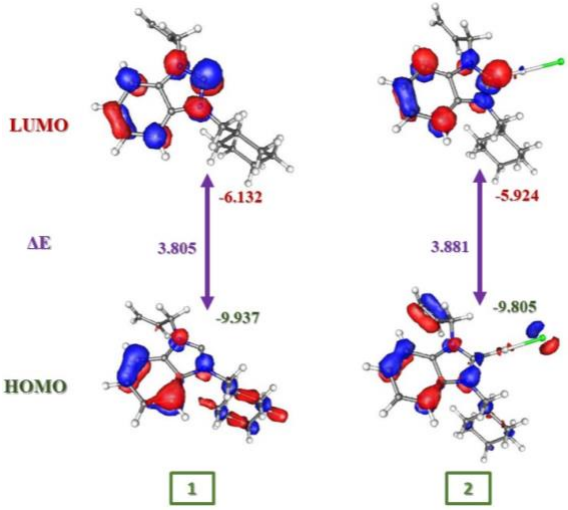


Figure 4. HOMO and LUMO Energies and Illustrations of **1** and **2** (in eV)

HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) of both molecules were analyzed by using the results that were obtained from DFT-based calculation methods. HOMO gives information about the place and tendency of the electron accepting properties of the molecules, while LUMO gives information about chemical reactions in which the molecules act as electron-donor (Golding Sheeba et al, 2021; Khajehzadeh and Sadeghi 2018). According to the results illustrated in Figure 4, LUMO of both NHC ligand and silver complex are located in the benzimidazole region. On the other hand, the HOMO of the NHC precursor is located on benzimidazole and cyclohexane, while the LUMO of the silver complex is located on the allyl and halogen regions. In addition, the calculated HOMO and LUMO energies were used to guess ionization potential (IP), electron affinity (EA) and electronegativity (χ) of the molecules (Table 1). The IP, EA and χ values of the ligand were calculated higher than complex (Kar et al, 2007). Global softness (S) and chemical hardness (η) are other criteria that give foresight about the reactivity properties of molecules. The reactivity of a molecule increases with decreasing the global softness of

a molecule. Global softness is the reciprocal of chemical hardness, and the higher the hardness, the lower the reactivity. According to the results, it can be noted that the ligand molecule is more reactive than the complex. The electrophilicity index (ω) is considered as an indicator of the electrophilic strength of the molecular system against a nucleophile (Parthasarathi et al, 2004). Therefore, the ligand is also more electrophile than the complex.

Table 1. Global reactivity descriptors of the complexes (in eV)

	1	**2**
Ionization Potential (IP)	9.937	9.805
Electron Affinity (EA)	6.132	5.924
Electronegativity (χ)	8.034	7.864
Global Softness (S)	0.263	0.258
Global Hardness (η)	1.902	1.940
Electrophilicity Index(ω)	16.975	15.955

Electronic transitions of optimized molecules were investigated by TDDFT based calculation methods with ORCA software (Neese, 2012). Allowed transitions with an oscillator strength greater than 0.01 are listed in Table 2.

Table 2. Energies (in nm), Oscillator Strength (f_{osc}), Main Orbital Contributions, and Type of Transition Involved in the Most Important Singlet Excitations for **1** in Gas Phase Calculated with TDDFT/BP86

state	λ / nm	f_{osc}	main transitions
		1	
31	271.9	0.0133	HOMO-4 → LUMO+1 (20.0%)
35	260.1	0.0204	HOMO-2 → LUMO+1 (13.2%)
36	256.9	0.0171	HOMO-1 → LUMO+1 (40.1%)
		2	
19	447.8	0.0414	HOMO-2 → LUMO (45.9%)
28	324.5	0.0152	HOMO-2 → LUMO+1 (33.4%)
			HOMO-3→ LUMO+1 (33.0%)
37	296.3	0.0148	HOMO-4 → LUMO+1 (19.3%)

VEGFR-2 triggers signaling pathways and plays more important role on angiogenesis than VEGFR-1 and the activation of VEGFR-2 could manage some pathologies of many diseases (Ghosh et al, 2008). In order to have foresight about the bioactivities of these molecules, the interactions with VEGFR-2 were analyzed by molecular docking methods. The binding affinity of the optimized ligand

with the VEGFR-2 crystal structure was determined as -6.49 kcal/mol, while the affinity of the silver complex was calculated as -5.06 kcal/mol. The amide-pi stacked interaction of the ligand with Cys1043 with is noteworthy. On the other hand, the H-bond of complex with Cys917 could be described the most important interaction. The alkyl, pi-alkyl, and van der Waals interactions were had influence the binding affinity of both molecules (Table 3 and Figure 5).

Table 3. Molecular docking results of the molecules for VEGFR-2 target

Molecules	Bind. Aff.*	Amino Acids Residue
		VEGFR-2 (1ywn)
1	-6.49	Cys1043 (Amide-Pi Stacked), Ile886, Val896, Leu1017, Cys1022, His1024 (Alkyl and Pi-Alkyl), Glu883, Leu887, Val897, Ile890, Ile1023, Ile1042, Asp1044 (van der Waals)
2	-5.06	Cys917 (Carbon Hydrogen Bond), Leu838, Ala864, Leu1033 (Alkyl and Pi-Alkyl), Val846, Val914, Glu915, Phe916, Lys918, Gly920 (Van der Waals)

* Binding Affinity in kcal/mol.

Sayed et al. evaluated the inhibitory potentials against VEGFR-2 for fifteen novel sulfonamides provided hydrazone-coupled derivatives and recorded good inhibitory activity. Schepetkin et al analyzed some oxime groups by molecular docking methods against VEGFR-2 for inhibition activity of tumor growth and metastasis and determined remarkable results. Also, El-Adl et al designed and synthesized ovel series of 5-benzylidenethiazolidine-2,4-dione derivatives and the interactions of these molecules with VEGFR-2 were recorded with good inhibitory activities. Additionally, new benzimidazolium-derived PEPPSI complexes were synthesized and characterized by Serdaroğlu et al. This group analyzed molecules by the molecular docking method with VEGFR-2 for their anticancer potential and −8.68 kcal/mol of the best binding affinity were recorded for a molecule. The results that calculated for this study, with compared to previous study, are remarkable.

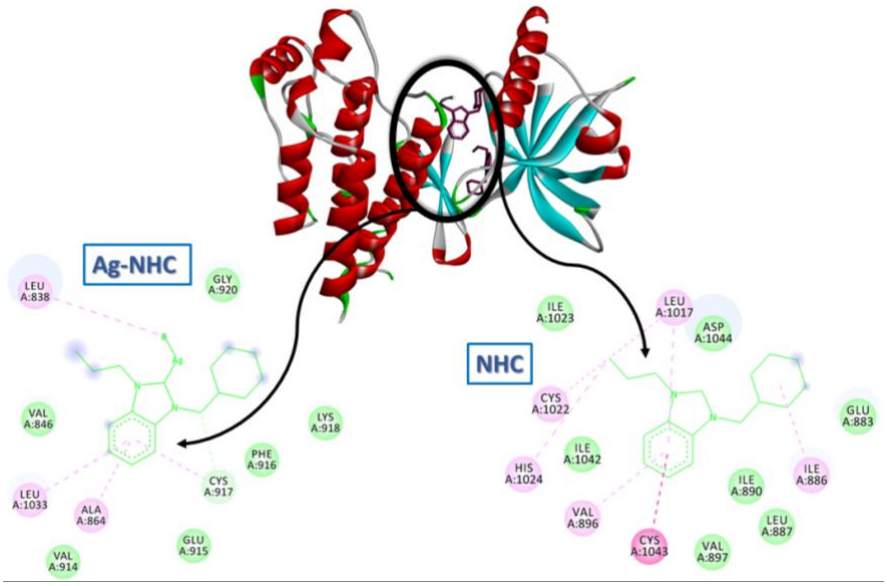


Figure 5. Interactions of the molecules with VEGFR-2 acquired by molecular docking

In this study, the interactions of molecules with DNA were also investigated using by molecular docking methods. It has been determined that each optimized molecule interacts with approximately the same region of the DNA crystal. DNA binding was detected between the ligand and Ade6, Thy7 and Thy8 aminoacids of DNA, while silver complex was interacted with Thy7, Thy8 (Figure 6).

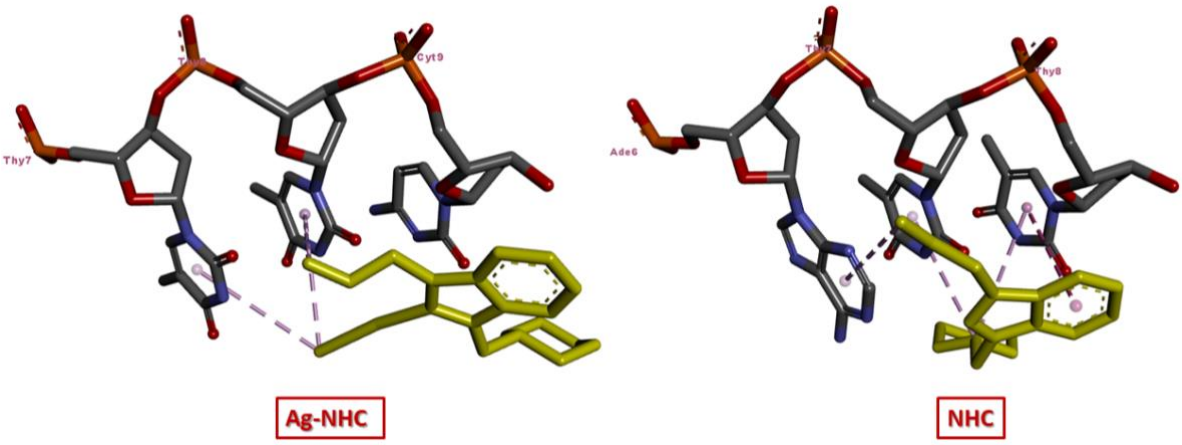


Figure 6. Molecular docking interaction of the molecules with DNA

4. Conclusions

NHC molecules and their metal complexes that have been analyzed for many activities attract the attention of the chemistry world. In this study, a new NHC molecule and its silver complex were synthesized and characterized. Spectroscopic and theoretical characterization of these molecules has also been added to the study. After performing frontier orbital analyzes of the optimized molecules, possible anticancer activities were analyzed by using molecular docking methods. The molecules had remarkable binding affinity with both VEGFR-2 and DNA. It is clear that these effects must analyzed more detailed, and the studies must be diversified with different substituted species. In future studies, we want to study with different substitution species and metals.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

References

Aher, S. B., Muskawar, P. N., Thenmozhi, K., and Bhagat, P. R. (2014). Recent developments of metal N-heterocyclic carbenes as anticancer agents. *European Journal of Medicinal Chemistry*, 81, 408-419.

Becke, A. D. (1988). Density-functional exchange-energy approximation with correct asymptotic behavior. *Physical review A*, 38(6), 3098.

Carmeliet, P., & Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature*, 407(6801), 249-257.

Choudhary, V., Bhatt, A., Dash, D., and Sharma, N. (2019). DFT calculations on molecular structures, HOMO–LUMO study, reactivity descriptors and spectral analyses of newly synthesized diorganotin (IV) 2-chloridophenylacetohydroxamate complexes. *Journal of Computational Chemistry*, 40(27), 2354-2363.

Dias, S., Hattori, K., Zhu, Z., Heissig, B., Choy, M., Lane, W., ... and Rafii, S. (2000). Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. *The Journal of Clinical Investigation*, 106(4), 511-521.

Diez-Gonzalez, S., Marion, N., and Nolan, S. P. (2009). N-heterocyclic carbenes in late transition metal catalysis. *Chemical Reviews*, 109(8), 3612-3676.

Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R. E. (1981). Structure of a B-DNA dodecamer: conformation and dynamics. *Proceedings of the National Academy of Sciences*, 78(4), 2179-2183.

El-Adl, K., El-Helby, A. G. A., Sakr, H., Eissa, I. H., El-Hddad, S. S., and Shoman, F. M. (2020). Design, synthesis, molecular docking and anticancer evaluations of 5-benzylidenethiazolidine-2, 4-dione derivatives targeting VEGFR-2 enzyme. *Bioorganic Chemistry*, 102, 104059.

Ferrara, N. (2004). Vascular endothelial growth factor: basic science and clinical progress. Endocrine reviews, 25(4), 581-611.

Ferrara, N. (2009). Vascular endothelial growth factor. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 29(6), 789-791.

Folkman, J. (1984). Angiogenesis. *Biology of Endothelial Cells*, 412-428.

Fontes, J. V., Santos, I. A., Rosa, L. B., Lima, R. L., Jardim, A. C., Miguel, D. C., and Abbehausen, C. (2022). Antileishmanial and Anti-Chikungunya Activity of Cu (I)-N-Heterocyclic Carbenes. *ChemistrySelect*, 7(31), e202201560.

Ghosh, S., Sullivan, C. A., Zerkowski, M. P., Molinaro, A. M., Rimm, D. L., Camp, R. L., and Chung, G. G. (2008). High levels of vascular endothelial growth factor and its receptors (VEGFR-1, VEGFR-2, neuropilin-1) are associated with worse outcome in breast cancer. *Human Pathology*, 39(12), 1835-1843.

Golding Sheeba, G., Usha, D., Amalanathan, M., Sony Michael Mary, M., and MarshanRobert, H. (2021). Molecular structure, vibrational spectroscopic, frontier molecular orbital and natural bond orbital analysis of anti-cancer drug 6-chloro-3-pyridine carbonitrile. *Spectroscopy Letters*, 54(6), 419-436.

Hamdi, N., Slimani, I., Mansour, L., Alresheedi, F., Özdemir, I., and Gürbüz, N. (2021). Rhodium (I) complexes with N-heterocyclic carbene ligands: synthesis, biological properties and catalytic activity in the hydrosilylation of aromatic ketones. *Journal of Coordination Chemistry*, 74(15), 2558-2579.

Herrmann, W. A. (2002). N-heterocyclic carbenes: a new concept in organometallic catalysis. *Angewandte Chemie International Edition*, 41(8), 1290-1309.

Herrmann, W. A., and Köcher, C. (1997). N-Heterocyclic carbenes. *Angewandte Chemie International Edition in English*, 36(20), 2162-2187.

Hoeben, A. N. N., Landuyt, B., Highley, M. S., Wildiers, H., Van Oosterom, A. T., and De Bruijn, E. A. (2004). Vascular endothelial growth factor and angiogenesis. *Pharmacological Reviews*, 56(4), 549-580.

Hopkinson, M. N., Richter, C., Schedler, M., and Glorius, F. (2014). An overview of N-heterocyclic carbenes. *Nature*, 510(7506), 485-496.

Kar, R., Chandrakumar, K. R. S., and Pal, S. (2007). The influence of electric field on the global and local reactivity descriptors: reactivity and stability of weakly bonded complexes. *The Journal of Physical Chemistry A*, 111(2), 375-383.

Karaaslan, M. G., Aktaş, A., Gürses, C., Gök, Y., and Ateş, B. (2020). Chemistry, structure, and biological roles of Au-NHC complexes as TrxR inhibitors. *Bioorganic Chemistry*, 95, 103552.

Khajehzadeh, M., and Sadeghi, N. (2018). Molecular structure, the effect of solvent on UV–vis and NMR, FT–IR and FT–Raman spectra, NBO, frontier molecular orbital analysis of Mitomycin anticancer drug. *Journal of Molecular Liquids*, 256, 238-246.

Koopmans, T. (1934). Über die Zuordnung von Wellenfunktionen und Eigenwerten zu den einzelnen Elektronen eines Atoms. *Physica*, 1(1-6), 104-113.

Miyazaki, Y., Matsunaga, S., Tang, J., Maeda, Y., Nakano, M., Philippe, R. J., ... and Nolte, R. T. (2005). Novel 4-amino-furo [2, 3-d] pyrimidines as Tie-2 and VEGFR2 dual inhibitors. *Bioorganic & Medicinal Chemistry Letters*, 15(9), 2203-2207.

Neese, F. (2012). The ORCA program system. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, 2(1), 73-78.

Neese, F. (2022). Software update: The ORCA program system—Version 5.0. *Wiley Interdisciplinary Reviews: Computational Molecular Science*, 12(5), e1606.

Neese, F., Wennmohs, F., Becker, U., and Riplinger, C. (2020). The ORCA quantum chemistry program package. *The Journal of Chemical Physics*, 152(22), 224108.

Nishida, N., Yano, H., Nishida, T., Kamura, T., and Kojiro, M. (2006). Angiogenesis in cancer. *Vascular health and risk management*, 2(3), 213.

Olofsson, B., Pajusola, K., Kaipainen, A., Von Euler, G., Joukov, V., Saksela, O., ... and Eriksson, U. (1996). Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proceedings of the National Academy of Sciences*, 93(6), 2576-2581.

Ott, I. (2017). Medicinal chemistry of metal N-heterocyclic carbene (NHC) complexes. In Inorganic and Organometallic Transition Metal Complexes with Biological Molecules and Living Cells (pp. 147-179). Academic Press.

Parthasarathi, R., Subramanian, V., Roy, D. R., and Chattaraj, P. K. (2004). Electrophilicity index as a possible descriptor of biological activity. *Bioorganic & Medicinal Chemistry*, 12(21), 5533-5543.

Perdew, J. P. (1986). Density-functional approximation for the correlation energy of the inhomogeneous electron gas. *Physical Review B*, 33(12), 8822.

Rahimi, N. (2006). VEGFR-1 and VEGFR-2: two non-identical twins with a unique physiognomy. *Frontiers in bioscience: a journal and virtual library*, 11, 818.

Rajkumar, P., Selvaraj, S., Suganya, R., Kesavan, M., Serdaroğlu, G., Gunasekaran, S., and Kumaresan, S. (2020). Experimental and theoretical investigations on electronic structure of 5-(hydroxymethyl)-2-furaldehyde: An antisickling agent identified from terminalia bellirica. *Chemical Data Collections*, 29, 100498.

Şahin, N., Çelebi, M. S., Ayvaz, M. Ç., and Üstün, E. (2022). Antioxidant Activity, Enzyme Inhibition, Electrochemical and Theoretical Evaluation of Novel PEPPSI Type N-Heterocyclic Carbene Complexes. *Inorganic Chemistry Communications*, 110028.

Şahin-Bölükbaşı, S., and Şahin, N. (2019). Novel Silver-NHC complexes: Synthesis and anticancer properties. *Journal of Organometallic Chemistry*, 891, 78-84.

Sayed, A. M., Taher, F. A., Abdel-Samad, M. R., El-Gaby, M. S., El-Adl, K., and Saleh, N. M. (2021). Design, synthesis, molecular docking, in silico ADMET profile and anticancer evaluations of sulfonamide endowed with hydrazone-coupled derivatives as VEGFR-2 inhibitors. *Bioorganic Chemistry*, 108, 104669.

Schepetkin, I. A., Plotnikov, M. B., Khlebnikov, A. I., Plotnikova, T. M., and Quinn, M. T. (2021). Oximes: Novel therapeutics with anticancer and anti-inflammatory potential. *Biomolecules*, 11(6), 777.

Serdaroğlu, G., Şahin, N., Üstün, E., Tahir, M. N., Arıcı, C., Gürbüz, N., and Özdemir, İ. (2021). PEPPSI type complexes: Synthesis, x-ray structures, spectral studies, molecular docking and theoretical investigations. *Polyhedron*, 204, 115281.

Torres, P. H., Sodero, A. C., Jofily, P., and Silva-Jr, F. P. (2019). Key topics in molecular docking for drug design. *International Journal of Molecular Sciences*, 20(18), 4574.

Tripathi, A., and Misra, K. (2017). Molecular docking: A structure-based drug designing approach. *JSM Chem*, 5(2), 1042-1047.

Trott, O., and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455-461.

Üstün, E., and Şahin, N. Molecular Docking and DFT Analysis of Methallyl Substituted N-Heterocyclic Carbene Salts for Potential Anticancer Activity. *Ordu Üniversitesi Bilim ve Teknoloji Dergisi*, 11(2), 186-192.

Üstün, E., Demir, S., Coşkun, F., Kaloğlu, M., Şahin, O., Büyükgüngör, O., and Özdemir, İ. (2016). A theoretical insight for solvent effect on myoglobin assay of W (CO) 4L2 type novel complexes with DFT/TDDFT. *Journal of Molecular Structure*, 1123, 433-440.

Wei, B. Q., Baase, W. A., Weaver, L. H., Matthews, B. W., and Shoichet, B. K. (2002). A model binding site for testing scoring functions in molecular docking. *Journal of Molecular Biology*, 322(2), 339-355.