Synthesis, Structural Calculations and Molecular Docking Studies of a Novel Uracil Derived Organic Molecule

Yeni Urasil Türevi Organik Bir Molekülün Sentezi, Yapısal Hesaplamaları ve Moleküler Modelleme Çalışmaları

Research Article

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ABSTRACT

A novel uracil derived compound, (E)-5-((3-chloro-2-hydroxybenzylidene)amino) pyrimidine-2,4(1H,3H)-dione, was synthesized and characterized using spectroscopic techniques. The interaction of the molecule with DNA was explored using computational methods which revealed that the molecule could act as a groove binder. The physicochemical properties of the molecule such as frontier molecule orbitals and chemical reactivity parameters were also investigated.

Key Words
Uracil, Schiff base, DNA binding, groove binder, molecular modelling.

ÖZ

Urasil türevi yeni bir bileşik, (E)-5-((3-klor-2-hidroksibenziliden)amino)pirimidin-2,4 (1H,3H)-dion, sentezlendi ve spektroskopik yöntemler kullanılarak yapı tayini gerçekleştirildi. Molekülün DNA ile olan etkileşimi, bilgisayar destekli yöntemler ile araştırıldı ve oluk bağlayıcı olarak davranabileceği belirlendi. Bileşiğin, molekül orbitalleri ve kimyasal reaktivite parametreleri gibi fizikokimyasal özellikleri incelendi.

Anahtar Kelimeler
Urasil, Schiff bazı, DNA bağlanma, oluk bağlayıcı, moleküler modelleme.

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INTRODUCTION

DNA has a critical role in recent drug development processes. As it is responsible for both replication and transcription, DNA interacting small organic molecules have a high potential as a drug candidate. There is a variety of biologically active molecule which is known to strongly interact with DNA and used for the treatment of cancer, AIDS and malaria [1]. The electrostatic interactions, intercalation and groove binding are the ways of small molecules to interact with DNA [2-6]. The groove binders such as 4,6-diamidino-2-phenyl-indole (DAPI) in Figure 1 are the molecules that bind to the minor or major grooves of DNA and play a key role in drug research.

Uracil is one of the four nucleobases in RNA. 5-aminouracil is an attractive molecule in terms of DNA binding studies as a result of its ability to form strong hydrogen bonds, multifunctional structure of lactim-lactam tautomer and functional group diversity which are all suitable for chemical derivatizations [7].

During our theoretical studies on the determination of uracil derived small organic molecules with high DNA binding affinity, we have found that a novel molecule (3-Cl-5-AAU) derived from 5-aminouracil and 3-chloro-2-hydroxybenzaldehyde has the potential to act as a groove binder. As a result, the molecule, 3-Cl-5-AAU, was synthesized, characterized and further investigated in terms of some structural features such as frontier molecular orbitals, electrostatic potential map, chemical reactivity and DNA interactions.

MATERIALS and METHODS

Chemistry

All reagents were obtained from commercial sources and used without further purification. Silica gel F_{254} (Merck 5554) precoated plates were used for thin layer chromatography. Infrared spectrum was recorded on a NICOLET - IS50 - FTIR. ^{1}H NMR and ^{13}C NMR spectra were carried out using a 400 MHz Bruker NMR spectrometer at ambient temperature. The elemental analysis was performed on a Costech ECS 4010 analyzer. Melting point was recorded with an electro thermal digital melting points apparatus.

Synthesis of (E)-5-((3-chloro-2-hydroxybenzylidene)amino)pyrimidine-2,4(1H,3H)-dione

5-aminouracil (112 mg, 1 mmol) and 3-chloro-2-hydroxybenzaldehyde (172 mg, 1.1 mmol) were placed in a flask with 20 ml ethanol and a few drops of glacial acetic acid. This mixture was refluxed for several hours until the reaction was completed. Then the yellow precipitate was filtered off, washed with hot ethanol and air dried.

Yield: 259 mg (97%); Mp: >250°C; IR (ATR): ν = 3194, 3069, 2911, 1709, 1663, 1595, 1448, 1424, 820, 779 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS) ν (ppm): 6.93 (t, J= 5.2 Hz, 1H), 7.46 (d, J= 5.2 Hz, 1H), 7.49 (d, J= 5.2 Hz, 1H), 7.98 (s, 1H), 9.48 (s, 1H), 11.49 (bs, 2H); ^{13}C NMR (400 MHz, DMSO-d_6, 25 °C, TMS): ν 118.9, 119.4, 120.0, 120.5, 130.8, 132.1, 138.0, 149.9, 155.6, 160.3, 160.9. Anal. Calcd. for C11H8ClN3O3: C, 49.73; H, 3.04; N, 15.82%. Found: C, 49.70; H, 3.04; N, 15.81%.

DFT Studies

All quantum chemical calculations in this work were carried out using Gaussian 09 software packed [8]. The structures of the molecule (3-Cl-5AAU) was optimized using density functional

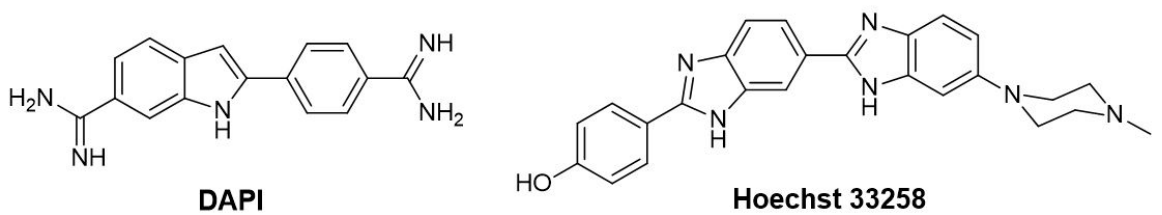


Figure 1. Chemical structure of some DNA groove-binding agents.

theory (DFT) with the Becke–Lee–Yang–Parr functional (B3LYP) method [9] with 6-311++G(d,p) basis set. Harmonic frequencies of the structures were calculated at the same method and basis sets to find local minima. The optimized structural parameters were used for all of the docking and physicochemical calculations in this study.

Chemical Reactivity

In order to obtain a detailed information about reactivity of 3-Cl-5-AAU, chemical reactivity parameters, chemical hardness (η) [10], chemical potential (μ) [10], and electrophilicity index (ω) [11] were evaluated from HOMO and LUMO energies. On the other hand, dipole moment is a useful index for the description of interactions between two chemical species. Therefore, the larger the dipole moment is, the stronger the van der Waals interaction will be. The chemical hardness (η) and the chemical potential (μ) are fundamental indicators of the stability and overall reactivity of a chemical system. The electrophilicity index (ω) allows quantitative description of the global electrophilic or nucleophilic nature of chemical species, physically signifying the propensity of chemical species to accept electrons.

Molecular Docking

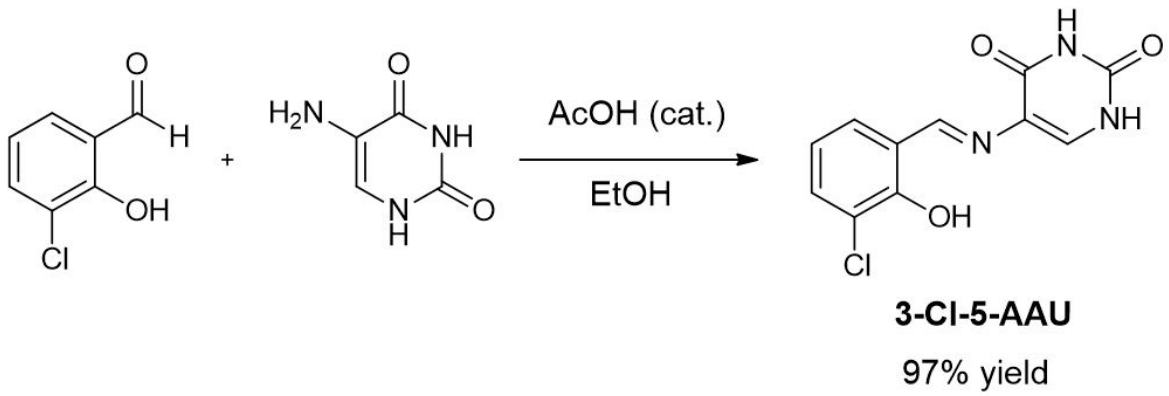
Molecular docking studies were performed using Autodock Vina program [12]. The crystal structure of DNA was obtained from the Protein Data Bank (PDB ID: 1BNA). The PDB format of 3-Cl-5-AAU was obtained by converting its 'out' files using Autodock software. Docking was performed to find the most stable and favorable orientation. Visualization of connected systems was performed using Discovery Studio 3.5 software. The binding sites were centered on the DNA, and a grid box was created with 60 × 60 × 60 points and a 0.375 Å grid spacing in which almost the entire macromolecule was involved. All other parameters were kept at their default values.

RESULTS and DISCUSSION

The target molecule, 3-Cl-5-AAU, was obtained in high yield with a simple acetic acid catalyzed Schiff base reaction between 5-aminourasil and 3-chloro-2-hydroxybenzaldehyde in ethanol (Scheme 1). The structure of 3-Cl-5-AAU was fully characterized using spectroscopic methods (FTIR, ^{1}H NMR and ^{13}C NMR) and elemental analysis.

The most stable molecular structure of 3-Cl-5-AAU was optimized by the DFT/B3LYP method with 6-311++G(d,p) basis set. The bond parameters (bond lengths and angles) of the molecule are listed in Table 1. The optimized structure with numbering of atoms is shown in Figure 2.

Generally, the bond parameters calculated with 6-311++G(d,p) basis set are very close to experimental values for small organic molecules in the literature [13]. The most important bonds of uracil compounds are the two carbonyl (C1–O1 / C2–O2) double bonds. The bond lengths of these carbonyl groups were calculated at ca. 1.212 and 1.218Å. In addition, the N-H bonds of the molecule were performed at 1.010Å. The imine bond length (C5–N3) was calculated at ca. 1.297Å. The C-C bond lengths of the phenyl ring were in the ranges



Scheme 1. Synthesis of 3-Cl-5-AAU

Table 1. Selected geometric parameters of 3-Cl-5-AAU molecule.

Bond lengths (Å)	DFT	Bong angles (°)	DFT
C1-O1	1.211	O1-C1-N2	124.4
C1-N2	1.384	C1-N2-C2	128.9
C2-N2	1.406	N2-C2-O2	119.6
C2-O2	1.218	C4-C3-N3	117.1
C2-C3	1.475	C3-N3-C5	124.4
C3-C4	1.362	C5-C6-C7	118.9
C4-N1	1.368	C6-C7-C8	121.1
C3-N3	1.393	C9-C10-C11	121.1
C5-N3	1.296	C10-C11-O3	119.7
C5-C6	1.452	C11-O3-H	106.7
C6-C7	1.406	C9-C10-Cl1	119.9
C9-C10	1.387		
C11-O3	1.337		
O3-H	0.993		
C10-Cl1	1.751		
N1-H, N2-H	1.010		

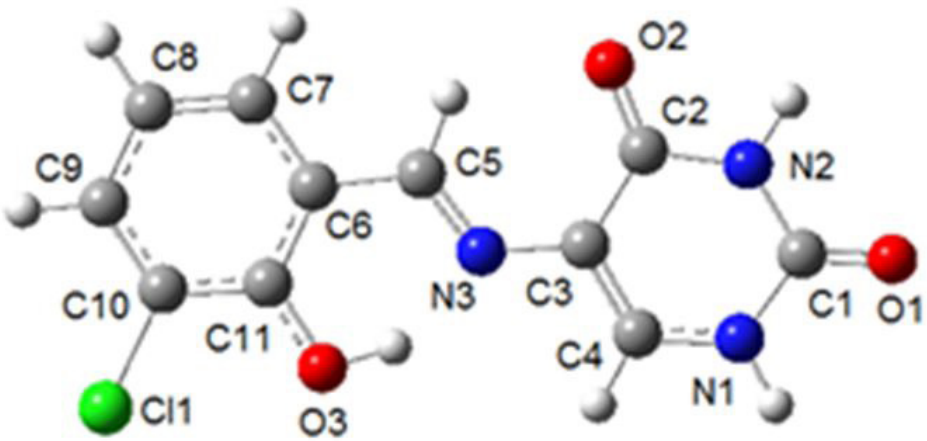


Figure 2. The most stable optimized molecular structure of 3-Cl-5-AAU.

of 1.387-1.406Å while the C10-Cl bond length was calculated at ca. 1.751Å. All atoms were on the same plane, that is, the molecule is planar.

Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) are very significant parameters to determine reactivity of molecules. The frontier molecular orbital (HOMO and LUMO) shapes of 3-Cl-5-AAU were determined using DFT/B3LYP method with 6-311++G(d,p) basis set. The energy band gap between the HOMO and LUMO for this molecule is given in Figure 3.

The calculated HOMO and LUMO energies of the molecule were -6.392 and -2.502 eV, respectively, and thus, the energy difference was 3.890 eV. Using the HOMO and LUMO energy

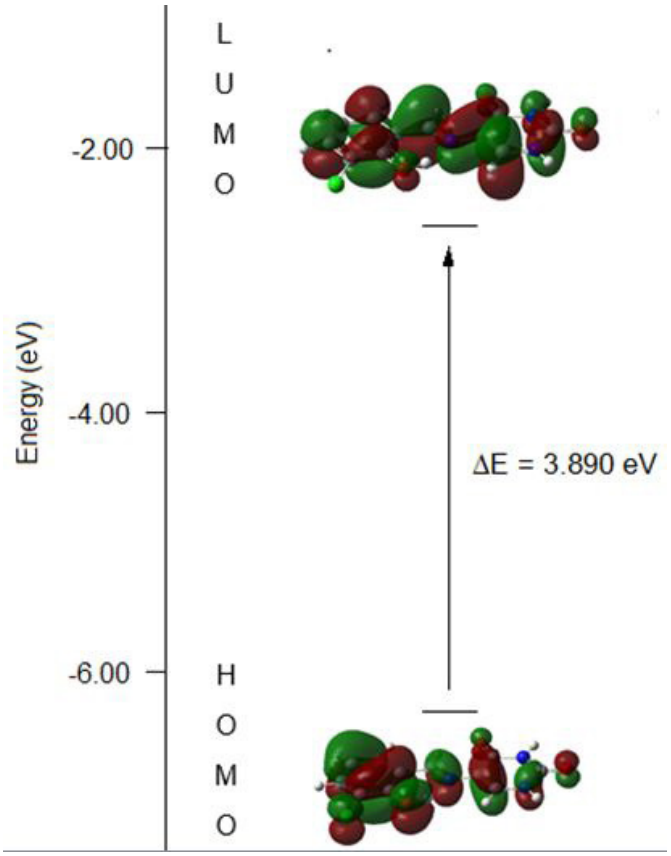


Figure 3. Frontier molecular orbitals of 3-Cl-5-AAU with the energy gap.

values, the global chemical reactivity descriptors of the molecule are defined as: hardness, η = (I-A)/2 = 1.945, electronegativity, χ = (I+A)/2 = -4.447, softness S = 0.257 and electrophilicity index $\omega = \mu^2 / 2\nu$ = 5.084 where A ($-E_{LUMO}$) and I ($-E_{HOMO}$) are the ionization potential and electron affinity. These results are presented in Table 2. Such quantities have been used to understand toxicity in terms of reactivity and site selectivity [14].

The reactive behavior of the molecule is visualized with the help of three-dimensional molecular electrostatic potential (MEP) surface. The surfaces of the molecule was plotted over an optimized electronic structures using B3LYP/6-311++G(d,p) as shown in Figure 4.

Molecular electrostatic potential (MEP) is used for investigating the chemical reactivity of a molecule and represented by different colors. The maximum negative region with preferred site for electrophilic reactivity is indicated by blue and nucleophilic reactivity is indicated by yellow [15]. The MEP map of 3-Cl-5-AAU illustrates that the negative potential sites in the whole molecule are on protons attached to the nitrogen atoms of the uracil ring and also mainly on the N1 proton. These theoretical calculations are consistent both with spectroscopic and experimental results. As the electron density around a nucleus decreases, it is said to be deshielded and resonates at higher ppm values in a ^{1}HNMR experiment. The NH protons of 3-Cl-5-AAU molecule are also deshielded and resonated at 11.49 ppm. These theoretical calculations are also compatible with some experimental studies related to reactivity of uracil derivatives in N-alkylation reactions [16]. According to those studies, N1 atom of uracil was easily alkylated first and protecting group strategy was used in the need of selective N2 alkylations.

There has been increasing interest in the usage of docking methods to study the binding of molecules to DNA. The structure of 3-Cl-5-AAU was optimized using density functional theory (DFT) with the Becke–Lee–Yang–Parr functional

Table 2. HOMO and LUMO energies, the energy gap (ΔE), absolute electronegativity (χ), chemical potential (μ), chemical hardness (η), softness (S) and electrophilicity index (ω) of 3-Cl-5-AAU using B3LYP/6-311++G(d,p) level.

Global reactivity descriptors	3-Cl-5-AAU
E (HOMO, eV)	-6.392
E (LUMO, eV)	-2.502
ΔE (eV)	3.890
χ	-4.447
μ	4.447
η	1.945
S	0.257
ω	5.084

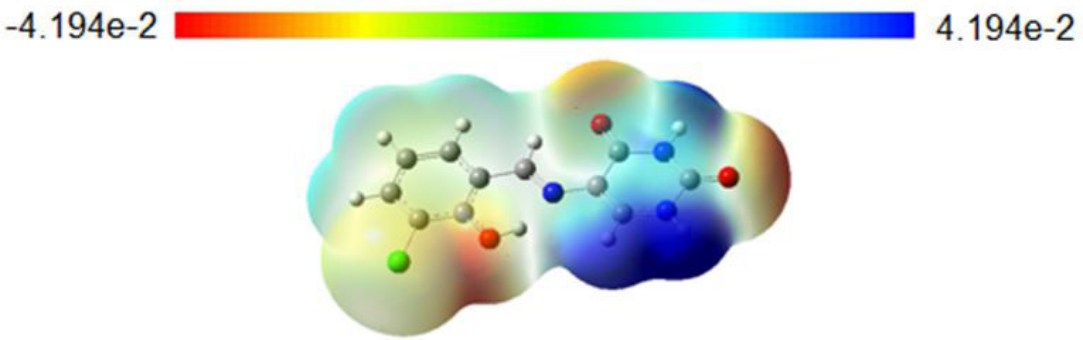


Figure 4. The total electron density mapped with molecular electrostatic potential surface of 3-Cl-5-AAU.

(B3LYP) method. Docking was performed to find the most stable and favorable orientation and the most favorable docked orientation is depicted in Figure 5.

The related values of hydrogen bonding interactions such as distance and free binding energy of the most stable docked orientation of 3-Cl-5-AAU was depicted in Table 3.

According to these results, 3-Cl-5-AAU molecule was mainly stabilized by three hydrogen bonding interactions and it moderately interacts with DNA adjacent to the G/C rich sequence of the minor groove. The binding free energy of the docked structure was computed to be 33.472 kJ.mol^{-1}.

In conclusion, a novel molecule, 3-Cl-5-AAU, was synthesized and fully characterized using different spectroscopic methods such as FTIR, ^{1}H NMR, ^{13}C NMR and elemental analysis. DFT calculations were performed to analyze the frontier molecular orbitals and chemical reactivity of the molecule. ***In silico*** methods were used to determine the binding potential of this molecule to DNA. The docking analysis revealed that the binding of the compound with DNA preferably takes place in the minor groove and the interaction strength through hydrogen bonding is encouraging for a small organic molecule. To the best of our knowledge, this is the first theoretical and experimental study related to the structural features of such uracil compounds. Design and synthesis of similar uracil derived compounds are under investigation in our laboratories for further ***in vitro*** activity studies.

Figure 5. Computational docking model illustrating the interactions between DNA (PDB code: 1BNA) and 3-Cl-5-AAU.

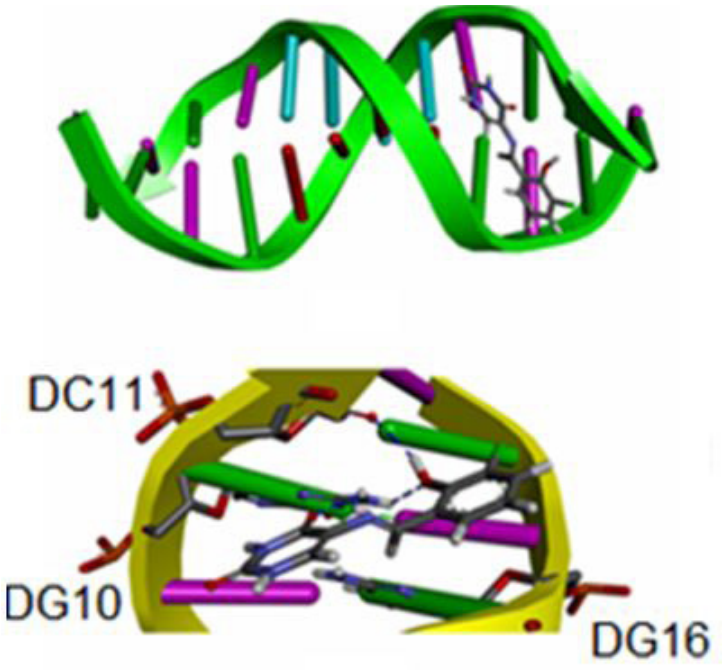


Table 3. Hydrogen bonding interactions and the binding free energy of the most stable docking conformation for 3-Cl-5-AAU docked into DNA.

Donor (D-H)	Acceptor (H××××A)	Distance (H××××A, Å)	ΔG_{bind}^{*} (kJ.mol^{-1})
O3H	O2 (DC11-DNA,chainA)	2.16	
N2H21 (DG10-DNA,chainA)	O3	2.40	-33.472
N2H22 (DG10-DNA,chainA)	O2	2.70	

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