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A Detailed Study of Solvent-Ligand Interactions and in Silico Biological Activity Predictions on Hydroxychloroquine

Mustafa Tuğfan BİLKAN

Highlights:

- Solvent effects on the structural properties of Hydroxychloroquine were investigated.
- Solvent-ligand complexes of HCQ have also been studied
- The drug-likeness, ADME, and toxicity parameters were examined in silico

Keywords:

- Solvent Effects
- In silico Predictions
- ADME-T
- Density Functional Theory

ABSTRACT:

In this study, the effects of solvent environment changes, which are of critical importance in drug production processes, on the geometric structure and physicochemical parameters of the Hydroxychloroquine (HQC) molecule were investigated. For this purpose, optimized molecule structures were obtained using Density Functional Theory in vacuum and solvent environments. Based on the optimized structures, the molecule's thermochemical properties, atomic charges, and chemical reactivity data were calculated in vacuum and solvent environments. Moreover, the molecule's molecular electrostatic potential map and HOMO-LUMO contour maps were drawn. Vibrational frequencies, intensities, and assignments in solvent environments were determined. The characteristics of the hydrogen bonding interactions established between solvent molecules and HQC were determined in detail. ADME, toxicity, and drug-likeness predictions of the molecule were made. The study results showed that while the structural, chemical, and physical properties of the HQC molecule were severely affected when transferred to the solvent environment, they were less affected by the changes between solvent environments. In addition, very strong h-bond interactions are established between the solvent molecules and HQC.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) started in late 2019 in Wuhan, China, one of the world's largest countries and trade centers. It has spread rapidly all over the world as a result of China's commercial and social interactions with other countries. According to WHO data on April 1, 2023, the virus caused more than 760 million people to be infected and nearly 7 million to die. (WHO Coronavirus Dashboard, 2023). Later, the disease, declared a pandemic by the WHO, showed severe effects, especially in people with weak immunity, chronic respiratory diseases, and older adults. The disease, which shows symptoms such as severe shortness of breath, high fever that cannot be reduced, and cough, can cause more severe health problems such as pneumonia, hypertension, gastrointestinal diseases, and coronary heart diseases in advanced stages (Hasgül et al., 2022; Acerce et al., 2022).

Recently, many pharmacological agents have been researched for treating the disease, and many drugs have been tried. One of the drugs used extensively for these studies is the Hydroxychloroquine (HCQ) molecule. HCQ is a critical antimalarial and antiviral agent on the WHO essential drugs list (WHO List of Essential Medicines, 2019). The other important medical uses of the drug include treating rheumatoid arthritis, lupus, and porphyria, also known as vampire disease (Hydroxychloroquine Sulfate Monograph for Professionals, 2020). A detailed literature review can reveal that many biological and chemical studies have been conducted on HCQ due to its acute pharmacological effects (Fox and Rheu, 1993; Ejuh et al., 2020; Noureddine et al., 2021; Altalhi et al., 2021; Parlak et al., 2022). When a search is done with the keyword "Hydroxychloroquine", it can be seen that between January 2020 and April 2023, there were 38700 results in Google Scholar and 5438 results in PubMed. This result shows that there is an increasing interest of scientists in HCQ. Much work has been done on the determination of the chemical and physical properties of the molecule using quantum chemical methods. In one of them, DFT and HF methods calculated chemical reactivity values and thermodynamic analyses of HCQ and CQ (Chloroquine) molecules (Omer et al., 2020). Another DFT study was carried out by Liu et al. in 2021 on determining vibrational modes and Fukui Indices of the molecules (Liu et al., 2021). In 2022, Chafai et al. calculated the chemical reactivity parameters and vibrational modes of HCQ and CQ molecules in the gas phase by DFT (Chafai et al., 2022). To the best of our knowledge, a detailed study of the effects of different solvent media on the structural, electronic, and spectroscopic parameters of HCQ has yet to be done. Solvent effects directly affect a drug's absorption, distribution, and transport in living tissues. In this respect, it is possible to increase the solubility and, thus, the drug's potency by using different solvents. Considering that most drugs used for therapeutic purposes have more or less undesirable side effects, it is vital to increase their effectiveness. Increased drug efficacy, which is generally directly proportional to its solvent effects, leads to lesser doses of medication, thus increasing the efficacy of the treatment and consequently reducing the side effects.

In this study, the effects of biologically and pharmacologically important solvent media such as ethanol (*EtOH*-Ɛ=*24.85*), dimethylsulfoxide (*DMSO*-Ɛ=*46.83*), and water (H_2O-Ɛ=*78.36*) on the structural, spectroscopic, electronic and thermochemical parameters of the HCQ molecule were investigated in detail. In addition, the hydrogen bonding interactions between HCQ and the solvent molecules are also discussed. The molecule's toxicity, drug-likeness, and ADME (Absorption, distribution, metabolism, and excretion processes) parameters were also calculated in silico. The results obtained were discussed and aimed to increase the beneficial use of the molecule.

MATERIALS AND METHODS

Gaussian 09 program was used for all parameters calculated using DFT in vacuum and solvent environments (Frisch et al., 2009). The Gaussview package program was used for drawing 3D structures and visualizing calculation results (Dennington et al., 2008). The 3D structure of HCQ in the gas phase and solvent media was drawn, and optimized geometries were determined. The molecule's structural, spectroscopic, and electronic parameters were calculated using optimized structures with DFT/B3LYP and 6-311++G(d,p) basis set. The fundamental vibrational modes were characterized with the VEDA4 program, using potential energy distributions (PED) (Jamróz, 2004). The counterpoise method described by Boys and Bernardi was used to determine the content of hydrogen bonding interactions (Boys & Bernardi, 1970). The ionization potential (*I*) and electron affinity (*A*) of the molecule were calculated as the negatives of the HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energies as stated in the Koopmans Theorem (Bilkan, 2019). Chemical potential ($\mu=(E_{HOMO}+E_{LUMO})/2$), chemical hardness ($\eta=(E_{LUMO}-E_{HOMO})/2$), and electrophilicity ($\omega=\mu^2/2\eta$) were obtained as defined by Parr et al. (Parr et al 1999). In the part of in silico biological activity calculations, the ADME and drug-likeness parameters were obtained using the PreADMET (PreADMET, 2022) website and toxicity parameters using the ProTox-II (ProTox-II, 2022) program. The interaction energies between solvent-ligand molecules were calculated using Formula 1 (Bilkan, 2017). In the formula, $E_{SOLVENT}$ is the energy of solvent molecules forming h-bonds with HCQ.

$$\Delta E_{INTERACTION}=E_{CLUSTER}-(E_{HCQ}+E_{SOLVENT}) \quad (1)$$

RESULTS AND DISCUSSION

The Optimized Structures and Thermochemical Parameters of HCQ in Solvent Media

Solvent media are very effective physical factors on the structure and all other properties of molecules. Solvents can change a molecule's geometric structure, charge distributions, thermochemical properties, and vibrational modes. These changing parameters are the determining factors in pharmacokinetic processes. Since the use of molecules as drugs is determined by the strength and quality of the chemical bonds they form with the active sites of proteins, the changing structural properties should also be examined in drug synthesis and production processes.

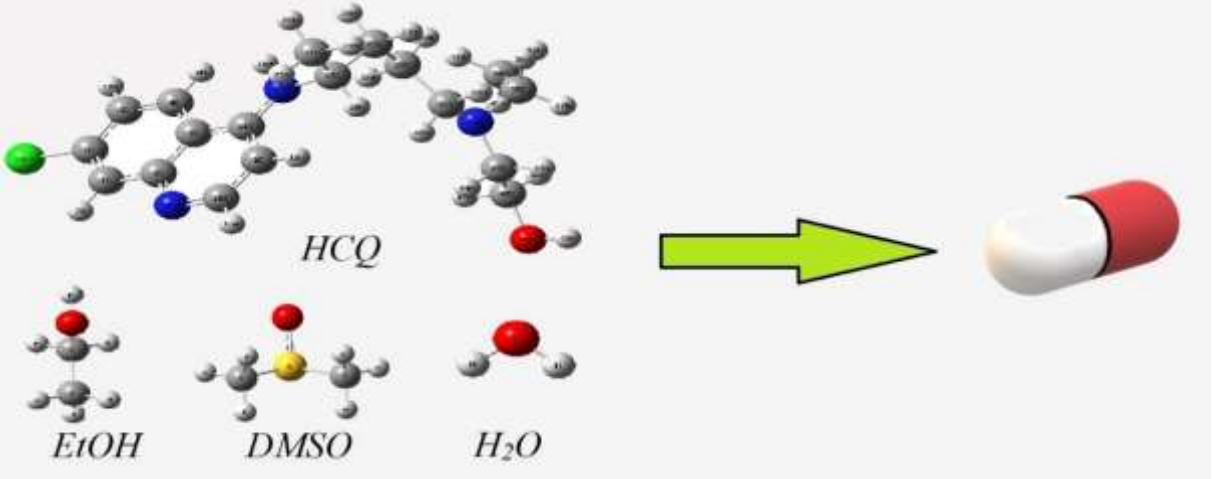

Figure 1. The Optimized Molecular Structures of HCQ, EtOH, DMSO, and Water

The fact that the human body is made up of around sixty-seventy percent of water makes it necessary to determine the extent to which molecular structures are affected by the aquatic

environment and the nature of the interactions between the drug and the water molecules. The optimized molecular structures of HCQ and the solvents are shown in Figure 1.

The total energies and thermochemical parameters of HCQ calculated in vacuum and solvent environments using optimized structures are shown in Table 1.

The increase in the dipole moment value is related to increased binding properties and can also increase a molecule's biological activity (Lien et al., 1982). Table 1 also shows that the thermochemical parameters of HCQ changed drastically from vacuum to solvent environments, but no significant differences were observed between solvent media.

Table 1. The Calculated Energies and Thermochemical Properties of HCQ in Solvent Media

	Vacuum	**EtOH**	**DMSO**	**H_2O**
ε (Dielectric constant)	1.00	24.85	46.83	78.36
Total Energy (Hartree)	-1401.528	-1401.545	-1401.546	-1401.546
Total Energy (Kcal/mol)	-879472.4	-879483.0	-879483.6	-879483.7
Energy Difference (Kcal/mol)	0.0	10.6	11.2	11.3
Zero-point vibr. Energy (Kcal/mol)	262.09	262.12	262.14	262.15
*E_0=E_{tot} + ZPVE (Hartree)	-1401.111	-1401.128	-1401.128	-1401.129
**E_{298}=E_{el} + E_{vib} + E_{rot} + E_{trans} (H)	-1401.086	-1401.104	-1401.104	-1401.105
***H_{298}= E_{298} + RT (Hartree)	-1401.085	-1401.103	-1401.103	-1401.104
****G_{298}= H_{298} – TS (Hartree)	-1401.168	-1401.185	-1401.185	-1401.185
Entropy (cal/mol.K)	174.587	172.632	172.212	172.016
Heat capacity (cal/mol.K)	90.727	90.698	90.684	90.675
Dipole Moment (Debye)	7.99	11.31	11.46	11.52

*E_0 : Sum of electronic and zero-point vibrational energy
** E_{298} : Sum of electronic and thermal energies
*** H_{298} : Sum of electronic and thermal enthalpies
****G_{298} : Sum of electronic and thermal free energies

For example, Table 1 shows the difference between the total energies in vacuum and solvent environments is 10.6 kcal/mol in EtOH, 11.2 kcal/mol in DMSO, and 11.3 kcal/mol in water media. There is no statistically significant difference between them. Similarly, dipole moments of HCQ in vacuum and the solvent media were calculated as 7.99 D, 11.31 D, 11.46 D, and 11.52 D, respectively. This may indicate that solvents other than water can also be used in biological studies and pharmacological research related to HCQ.

Atomic charges, molecular electrostatic maps, molecular contour maps and relations of these with h-bond ability of hcq in solvent media

The individual atomic charges of the atoms that make up a molecule are among the factors that determine all the physical, chemical and even biological properties of the molecule. Interatomic interactions and chemical bonds are formed as a result of the charge exchange between atoms or the attraction effects of atoms with each other. Therefore, determining the charge of each atom that makes up the molecule is among the important topics of computational quantum chemistry. Charges for each atom in the molecule can be calculated by Mulliken or Natural Bond Analysis (NBO) methods (Gangadharan & Krishnan, 2014). Although the results obtained by both methods provide very useful information, it is known that NBO-calculated atomic charges are more reliable than Mulliken charges (Yurdakul & Bilkan, 2015). In addition, NBO charge calculations are a very effective method for determining intermolecular interactions and provide useful information in studying charge transfers between molecular systems.

The NBO-calculated atomic charges of HCQ in vacuum medium are given in Figure 2. As can be seen from the figure, all nitrogen atoms and most carbon atoms have a negative charge. Such a

negative charge in atoms is very important for bioactivity because these negatively charged atoms form strong and weak bonds of bioactive molecules with proteins and other macromolecules. Although all hydrogen atoms in the molecule are positive as expected, the hydrogen atom bonded to the oxygen atom and those close to the chlorine atom have a higher positive charge density than the others. This directly affects the nature of hydrogen-bonded interactions that HCQ will form with solvent molecules. This result also indicates that these atoms are electron donors and charge transfer from H to C and from H to O. These partial charge distributions on the molecular structure show that electrostatic interactions between atoms can contribute significantly to intramolecular and intermolecular interactions.

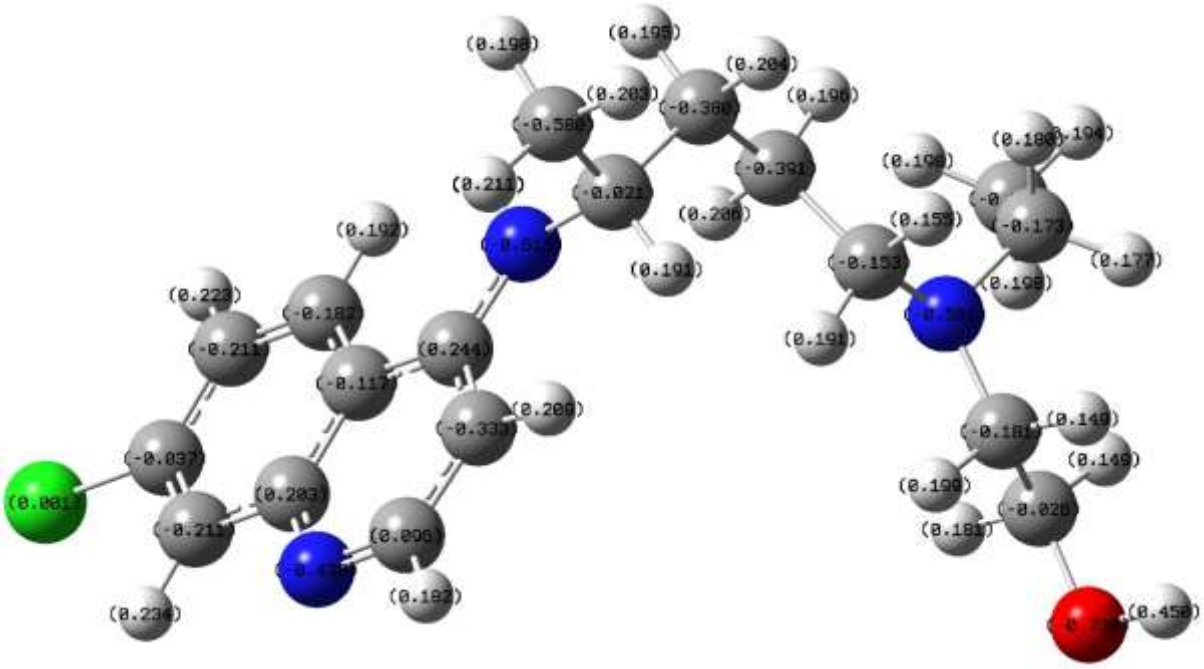

Figure 2. The NBO-Calculated Atomic Charges (in e) of HCQ in Vacuum

Figure 3 shows the molecular electrostatic potential map (MEP) of HCQ. MEP is directly related to the electron densities of atoms in a molecule. Therefore, it is an important parameter frequently used in determining the characteristics of hydrogen bonding interactions (Dege et al., 2022). Figure 3 shows the red, yellow, green, and blue regions on the map. The red-colored regions where the oxygen and nitrogen atoms are located are the ends of the molecule with the most negative potential. The strongest intermolecular interactions occur in these parts of the molecule. The blue-colored regions where the hydrogen atoms are located are the regions with the most positive potential. The green and yellow tones represent neutral or near-neutral charge densities.

From the MEP map, it is predicted that the oxygen and nitrogen ends of the HCQ are the regions that can establish the strongest hydrogen bonding interactions with solvent molecules. Taking advantage of this, the character of solvent-ligand interactions can be determined with higher accuracy and shorter computational times.

The HOMO and LUMO contour maps of HCQ are given in Figure 4. HOMO and LUMO energies are important parameters used to describe and calculate many important properties of molecular structures. Using HOMO and LUMO values, global reactivity descriptors such as polarity,

hardness, softness, basicity, acidity, excitability and structural indicators such as charge transfer and electronic transitions can be easily determined (Dege et al., 2022).

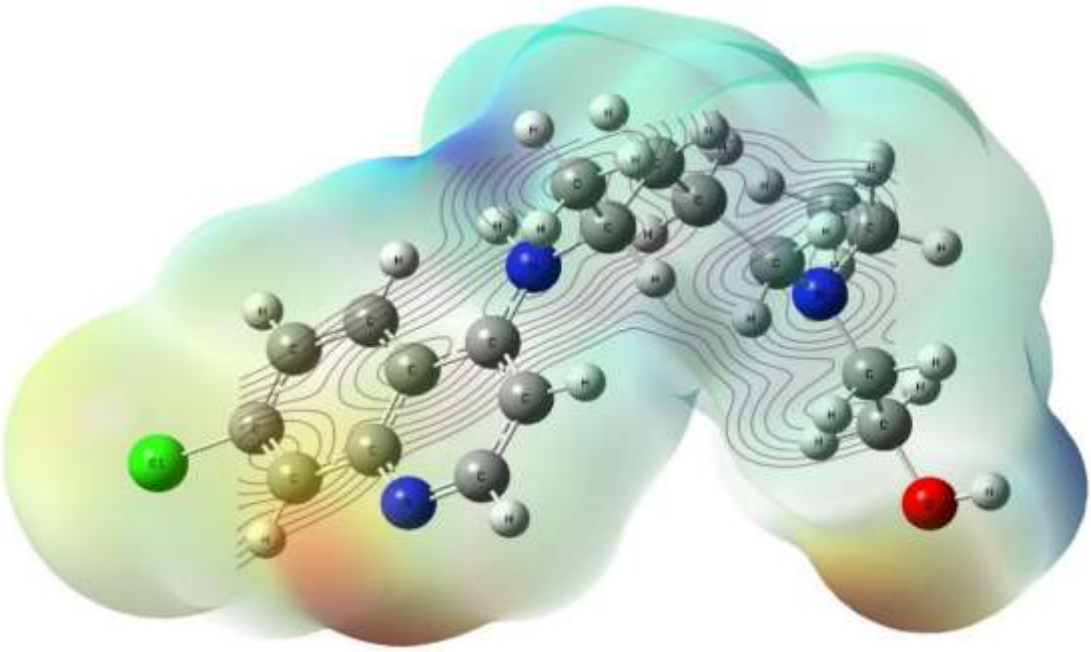

Figure 3. The Molecular Electrostatic Potential Map of HCQ

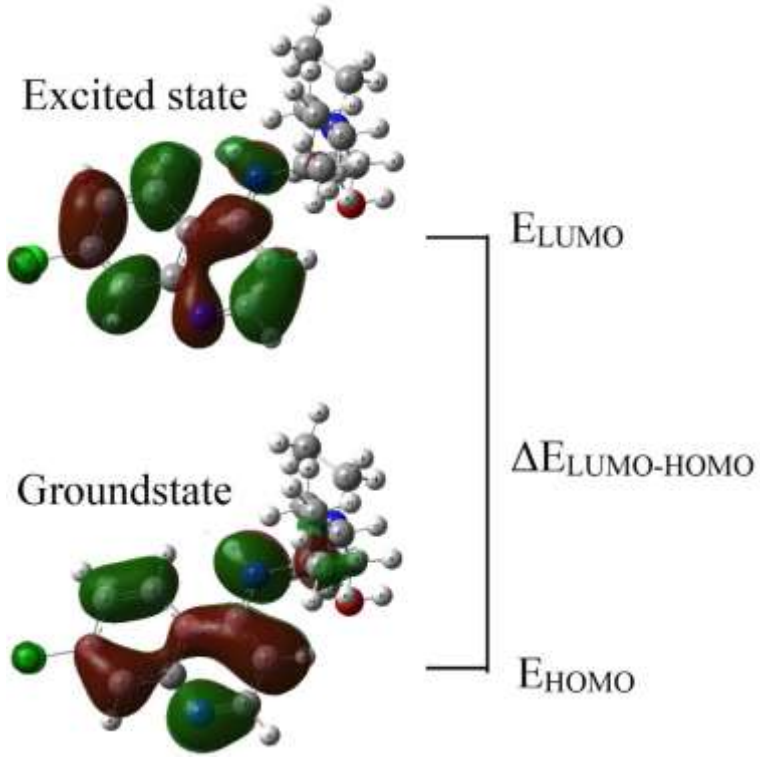

Figure 4. The HOMO-LUMO Contour Maps of HCQ

HOMO-LUMO and global reactivity values of HCQ calculated (Pearson, 1986; Üstün et al, 2016; Üstün, & Mehel, 2018) in vacuum and solvent environments are given in Table 2.

It can be seen from the table that the HOMO and LUMO energies calculated in the vacuum environment are -1.593 eV and -5.999 eV, respectively. The HOMO-LUMO energy gap, which is one

of the important indicators of molecular stability, was calculated as 4.406 eV. The table also shows the HOMO-LUMO energies calculated in solvent environments and the gap values between them.

Table 2. DFT Calculated the Chemical Reactivity Data of HCQ in Vacuum and Solvent Media

Parameters	Vacuum	EtOH	DMSO	Water
E_{LUMO}	-1.593	-1.676	-1.682	-1.685
E_{HOMO}	-5.999	-5.982	-5.985	-5.986
$\Delta E_{LUMO-HOMO}$	4.406	4.306	4.302	4.301
Electron affinity (A)	1.593	1.676	1.682	1.685
Ionization potential (I)	5.999	5.982	5.985	5.986
Global hardness (η)	2.203	2.153	2.151	2.150
Chemical potential (μ)	-3.796	-3.829	-3.833	-3.836
Electrophilicity (ω)	3.271	3.405	3.416	3.421

It is seen that the HOMO-LUMO energy gap decreases with increasing solvent polarity, and thus the molecular stability of HCQ increases. The table also shows that the electrophilicity value of the molecule increases in solvent environments. Electron affinity, another important calculated global reactivity descriptor, also increased in solvent environments. This is especially important in terms of the pharmacological use of HCQ.

Vibrational assignments

Vibrational spectroscopy can give very useful information in providing information about the presence of functional groups. For example, the presence of the same IR spectra for two samples whose structures or contents are unknown may indicate that these samples have similar or even the same compound structure. However, it does not provide information on the detailed structure of the molecular formula or structure. It mostly provides information about functional groups. Therefore, it is a limited technique used in conjunction with other analysis methods.

Atoms in a molecule make harmonic vibrational motions around their starting point. There are 3N-6 vibrational modes for a nonlinear molecule (Bilkan, 2017). Each of these modes creates absorption bands in a characteristic region. Considering this feature, examining molecular structures using vibration spectroscopy is a frequently used practical method. It is possible to obtain valuable information with vibrational spectroscopy, especially in investigating the effects of solvent environments on molecular structures.

Since the HCQ molecule consists of 49 atoms, it has (3x49)-6=141 vibrational modes. A comparison of some important vibration modes of HCQ calculated in solvent environments with experimental ones is given in Table 3.

When Table 3 is examined, it can be seen that solvent environments seriously affect the vibrational modes of HCQ. There are many red shifts and blue shifts in vibrational frequencies. The frequency of vibration (actually wavenumber) between the oxygen and the hydrogen atom to which it is attached was calculated at 3822 cm^{-1} for a single HCQ in the vacuum environment, while it shifted to 3810 cm^{-1} in the solvent environments. There was also an increase in the relative intensity of the vibrational mode. The most intensity vibrational mode1600 cm^{-1}, where C-C-C bending, N-C, and C-C stretching vibrations are seen for HCQ, shifted 1594 cm^{-1} with the effect of solvent medium. The table shows frequency shifts and intensity changes that occurred in other vibrational modes. This indicates that solvent environments seriously affect the structural and, thus, pharmacological properties of HCQ. Therefore, the effects of solvent media should be considered in detail in drug design studies related to HCQ.

Table 3. The Selected Vibrational Modes and Assignments of HCQ in Vacuum and Solvent Environments

Mode	DFT/B3LYP								PED (%)*
	Vacuum		EtOH		DMSO		Water		
	Freq.	IIR	Freq.	IIR	Freq.	IIR	Freq.	IIR	
22	285	20.98	282	13.39	282	13.71	282	14.18	$\Gamma_{HOCC}(40)$
28	399	11.25	399	11.23	401	12.16	402	12.57	$\delta_{CCN}(23)+\Gamma_{HNCC}(14)$
47	810	9.50	815	9.21	815	9.51	815	9.67	$\Gamma_{HCCN}(30)+\Gamma_{HCCC}(27)+\Gamma_{CNCC}(12)$
52	868	11.51	866	1.21	867	1.09	867	1.04	$\delta_{CCN}(16)$
54	906	12.14	901	10.86	901	10.77	901	10.73	$\delta_{CCC}(24)$
94	1402	18.41	1401	9.85	1401	4.96	1401	3.39	$V_{NC}(26)+\delta_{HCN}(15)+V_{CC}(13)$
113	1600	100.00	1594	100.00	1594	100.00	1593	100.00	$\delta_{CCC}(15)+V_{NC}(15)+V_{CC}(12)$
117	2877	33.23	2894	37.54	2895	37.61	2896	37.61	$V_{CH}(98)$
139	3207	0.13	3209	2.09	3209	2.04	3210	2.02	$V_{CH}(99)$
140	3623	4.04	3621	7.76	3621	7.89	3620	7.94	$V_{NH}(100)$
141	3822	6.67	3810	6.94	3810	7.04	3810	7.09	$V_{OH}(100)$

V: stretching, δ: bending, Γ:torsional

Solvent-ligand Complexes

Hydrogen-bonded (h-bond) complexes are one of the intriguing phenomena in computational quantum chemistry. Its importance is related to the direct effects of such binding on biological activity and bioavailability.

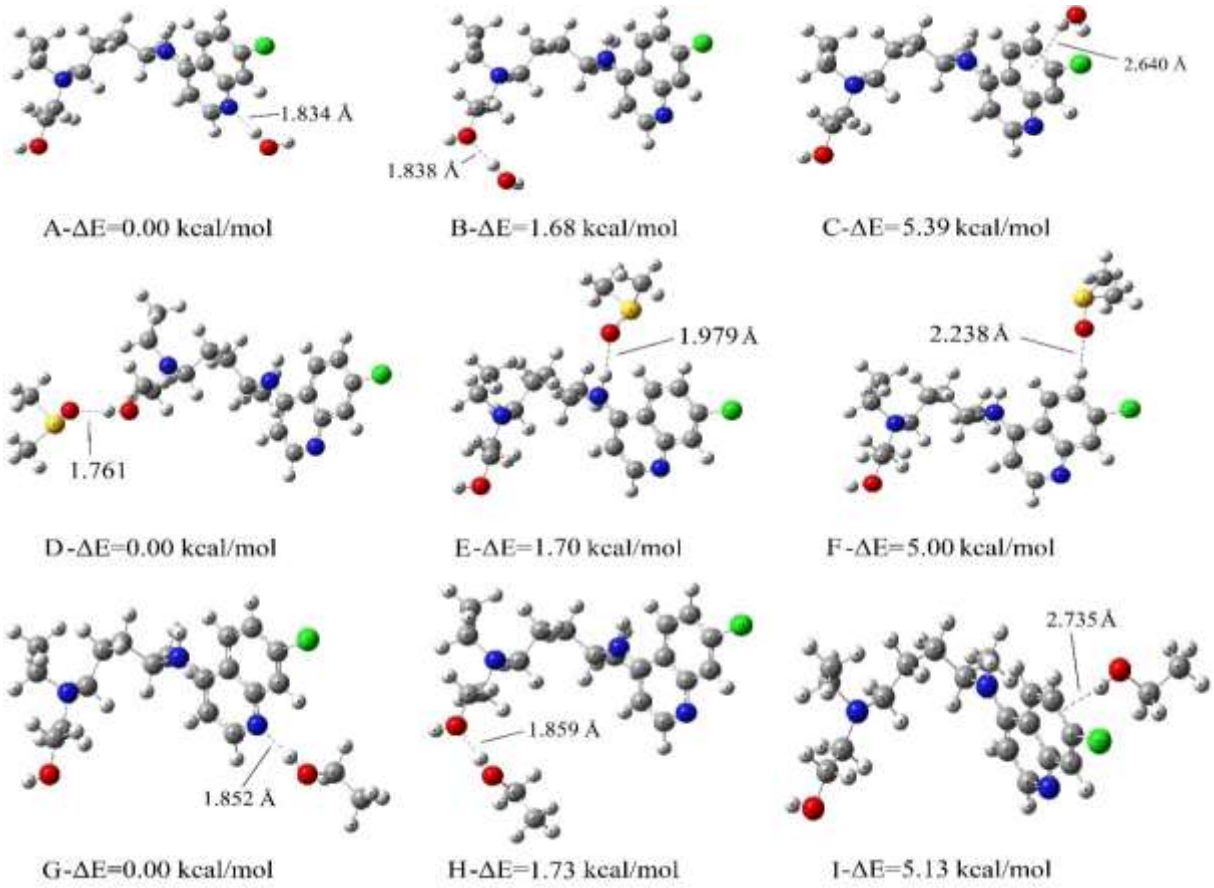

Figure 5. The Optimized Molecular Structures, Relative Energy Differences and H-Bond Lengths For Three Versions of HCQ and EtOH, DMSO, and Water Complexes

Drug-drug interactions, drug-solvent interactions, and even drug-protein interactions mostly occur due to hydrogen bonding mechanisms. H-bond formation is so crucial that it is first-degree effective in the emergence and continuity of life. H-bond effects are also seen in the formation of the structure of water, which gives life to almost all living things, the double-helix structure of the DNA macromolecule, and protein folding (Ghiandoni & Caldeweyher 2023). Here, the h-bonded solvent-ligand complexes of HCQ were investigated in terms of energies, h-bond length, and bond strength. Thus, the nature of the interactions between HCQ with water and other solvents was determined.

Optimized molecular structures of HCQ-H_2O, HCQ-EtOH, and HCQ-DMSO complexes are given in Figure 5, together with the binding distances and relative energy differences.

As can be seen from the Figure, HCQ contains a large number of h-bond donors, but only two h-bond accepters. In addition, h-π interaction occurs between the donors of the solvent molecules and the aromatic ring in the structure of HCQ. The hydrogen bond lengths formed as a result of the interactions vary between 1.731 and 2.735 Å. Among the hydrogen-bonded complexes formed, the most stable ones are indicated by A, D and G. The interaction energies for the HCQ-solvent complexes were calculated and given in Table 4.

Table 4. The Interaction Energies of HCQ-Solvent Complexes

Solvent media	Complex	Interaction Type	Interaction energy (kcal/mol)	H-bond lengths (Å)	Energy difference (kcal/mol)*
	A	O-H···N	-2.33	1.834	0.00
Water	B	O-H···O	-1.78	1.838	1.68
	C	O-H···π	-0.65	2.640	5.39
	D	O-H···O	-5.36	1.761	0.00
DMSO	E	N-H···O	-3.65	1.979	1.70
	F	C-H···N	-0.36	2.238	5.00
	G	O-H···N	-3.03	1.852	0.00
EtOH	H	O-H···O	-1.29	1.859	1.73
	I	O-H···π	-0.88	2.735	5.13

*Energy differences were calculated as A-A, A-B, and A-C for water complexes and others similarly

The intermolecular interaction energy is one of the most important indicators of the strength of non-covalent bonding. In stable complex structures, interaction energies (ΔE) are mostly negative, and the more negative this value is, the stronger the hydrogen bond will be (Zheng et al., 2016). As can be seen from the table, O-H···N and O-H···O type bonds are the bonding types with the strongest interaction energies. However, O-H···π type interactions are the weakest versions of hydrogen bonding. This means N and O atoms of HCQ will be donors in hydrogen bonding with solvent molecules. When a detailed literature study is conducted, it is seen that the interaction energy for HCQ-protein bindings is 5-10 kcal/mol in docking studies where the interactions of HCQ with various proteins are examined (Korkmaz et al., 2018; Amin & Abbas, 2021; Singh et al., 2021). This shows that the drug taken into the solvent media (or, for example, the human body) can easily interact with the proteins and make bindings from the active sites after being dissolved in the solvent environments examined in this study.

ADME, drug-likeness and toxicity parameters of HCQ

In medical and biological applications, there are some important parameters in applying a molecule with therapeutic effects to the patient as a drug. These parameters are absorption, distribution, metabolism, and excretion (ADME) processes, and the optimal benefit of the drug to the patient is directly related to these factors. In this respect, obtaining ADME data in pharmacokinetic processes can provide useful information at the preclinical stage of drug development because in silico

ADME calculations can show the potential of a molecule to be used as a drug (Butina et al., 2002; Cetin et al., 2023; Sevincli et al., 2023). Moreover, since a drug's absorption, distribution, metabolism, and excretion processes occur in solvent environments, ADME parameters and solvent effects are directly related. Due to its stated importance, in silico ADME predictions have serious importance (Cetin et al., 2023). The ADME parameters of the HCQ molecule were calculated in this study and given in Table 5.

Table 5. Some Important in Silico ADME Parameters of HCQ

ID	Value
BBB	2.29
Caco2	46.08
HIA	94.66
MDCK	45.11
Plasma Protein Binding	89.00
Pure water solubility (mg/L)	224.79

The table shows that the Blood-brain barrier penetration (BBB) value is 2.29. Molecules with a BBB value greater than 2.00 have high absorption (CN-active) in the central nervous system (Ma et al., 2005), indicating that HCQ can penetrate the BBB very well. The Caco2 (Lea, 2015) value is another important parameter, an immortalized cell line of human colorectal adenocarcinoma cells. This Cell line is used as a model of the intestinal epithelial barrier. Madin-Darby canine kidney (MDCK) cell isolated from canine distal kidney tissue has been used as a faster and more cost-effective alternative to Caco-2 cells. The Caco2 permeability value of HCQ was calculated as 46.08, and MCDK was calculated as 45.11. The HIA is a human intestinal absorption feature and indicates the absorption process of orally administered drugs from the gastrointestinal tract into the circulation. The calculated value of HIA is 94.66. All calculated parameters state that HCQ has a good oral absorption ratio in the human body.

Lipinski defines simple but very useful rules (Christopher, 2004) depending on the structural features to predict the use of a molecule as a drug. While these rules define the drug-likeness of the molecule, they are based on previous research, scientific studies, and reports. According to Lipinski, for a molecule to be easily absorbed, the H-bond donor number should be less than 5, the molecular weight should be less than 500, the logP should be less than 5, H-bond acceptor should be less than 10. In this respect, HCQ meets almost all requirements specified by Lipinski except for the number of H-bond donors.

It is important to detect the toxic effects of a drug or any substance to be taken into the body. Toxicity should not only be considered an effect on the whole body; it can also directly affect any organ or cell. Toxicity has some important features that differ from many other scientific concepts. First, toxicity is dose-dependent. There may be doses in which even substances with a very toxic effect are harmless. The second is species-specific. In other words, a substance with high toxicity may harm some living species while not causing others. Therefore, animal experiments are only sometimes possible in terms of toxicity. In this case, making toxicity estimations based on molecular structures can give useful and scientifically beneficial results. The predicted toxicity parameters of HCQ are given in Table 6.

It can be seen from the table that the LD_{50} value of HCQ is estimated at 1240 mg/kg. LD_{50} means *Median Lethal Dose*, which is frequently used in toxicity definitions (Anonymous, 2019). The

LD_{50} value of a toxic substance is the dose required to kill half of the population in a toxicity test, and the lower the LD50 value, the higher the toxicity of the substance (Anonymous, 2021).

Table 6. Some Important in Silico ADME Prameters of HCQ

ID	Value	Prediction
Predicted LD_{50} (mg/kg)	1240	-
Predicted Toxicity Class	4	harmful if swallowed
Hepatotoxicity	0.94	Inactive
Immunotoxicity	0.99	**Active**
Cytotoxicity	0.72	Inactive
Carcinogenicity	0.62	Inactive
Mutagenicity	0.79	**Active**

To make a comparison, the toxicity value for paracetamol, which is one of the most frequently used drugs in infants, children, adults, and elderly patients due to its antipyretic and analgesic effects, was calculated as 338 mg/kg. The experimental toxicity dose determined for paracetamol has been reported as approximately 150-200 mg/kg and above (Janssen & Saluja, 2015; Yoon et al., 2016). In addition, the toxicity class of both drugs is predicted to be 4 on the web server. However, it can be seen from the Table that HCQ is active in terms of immunotoxicity and mutagenic toxicity. In this case, it is recommended to consider these effects in the determination of the dosage of the drug and to apply treatments that will eliminate the negative effects on the immune system. All these results may show that the use of appropriate doses of HCQ in drug therapy will not cause toxic effects.

CONCLUSION

In this study, a detailed review of the effects of solvent media on HCQ was made. The situations of the molecule's physicochemical and pharmacological properties in EtOH, DMSO, and water environments were examined, and possible hydrogen-bonded compounds formed with solvent molecules were studied. In addition, drug-likeness, ADME, and toxicity estimates were made in silicon. The study results showed that the thermochemical parameters and dipole moment changed drastically when changing from vacuum to solvent environment, but there were no significant differences between solvent environments. The most negative parts of the molecule are oxygen and nitrogen atoms, while chlorine is almost neutral. Also, although all hydrogen atoms are positive, all but the one attached to the oxygen atom has a low charge density. Accordingly, HCQ has the potential to form hydrogen bonds with solvent molecules of oxygen, hydrogen, and all nitrogen atoms. In the MEP map, electron-excess regions are depicted in red and electron-deficient regions in blue. Thus, the nucleophilic and electrophilic properties of HCQ were interpreted, and versions with intermolecular interactions were determined. One of the important indicators of molecular stability, the HOMO-LUMO energy gap, is calculated as 4.406 eV in this study. In solvent environments, this gap is significantly reduced; that is, molecular stability is increased. In addition, while the electron affinity of the molecule increased in solvent environments, the global hardness decreased. Since the vibrational modes are directly related to the geometric structure, the frequencies and intensities also changed significantly due to the changing solvent environment. Some modes are red-shifted, while others are blue-shifted. The vibrations of atoms outside the ring are more severely affected by the solvent environment, while the vibrational modes within the ring are more limitedly affected. The h-bond length, interaction type, and interaction energies were also calculated for three solvent-ligand complexes formed with EtOH, DMSO, and water molecules. The highest interaction energies for EtOH and water compounds were calculated for O-H…N type bonds, and the h-bond lengths were

calculated as approximately 1.85 Å. For the DMSO complex, the h-bond length was calculated as 1.761 Å and the interaction energy as -5.36 eV. Finally, ADME, drug-likeness, and toxicity parameters of HCQ were also investigated in silico methods. Toxicity and ADME parameters were determined, and thus, preliminary basic information was established for future physical, chemical, biological, and pharmacological studies on HCQ.

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