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Investigation of Molecular Structures, Basicity Constants and their Interactions with Tumor-Associated Proteins of 2-((phenylimino)methyl)phenol Derivative Schiff Bases by Computational Methods

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ABSTRACT

HL1–HL5 Schiff bases were designed by attaching methyl, ethyl, isopropyl, and chloro substituents to the imine group of the 2-((phenylimino)methyl)phenol structure. The experimentally determined basicity constant of the Schiff bases were calculated at various computational levels and a comparative analysis was performed. The B3LYP/6-31G(d) level was determined as the optimal level. The geometries of HL1–HL5 Schiff bases were optimized at the B3LYP/6-31G(d) level. IR and NMR spectra were calculated. The ground state molecular structures of Schiff bases were characterized from IR stretching frequencies, ^{1}H-NMR and ^{13}C-NMR chemical shift values obtained from the optimized structures. Some molecular descriptors, basicity constants (pK_b), and proton affinities (PA) of the HL1–HL5 Schiff bases were calculated from aqueous-phase and gas-phase optimizations, respectively. It was found that there is an almost linear proportional relationship between the pK_b values of Schiff bases and their HOMO energies and proton affinities. Schiff bases were docked to protein chains with PDB codes 3HY3 and 1M17 in cell lines and their docking poses, binding energies (BE), interaction energies (IE) and total interaction energies (TIE) were calculated. To investigate whether there is a relationship between basicity strength and antitumor activity, BE, IE and TIE graphs were drawn against pK_b values of Schiff bases. It was found that the antitumor activity of Schiff bases with high pK_b values was generally high. However, it was determined that there was no linear relationship between pK_b value and antitumor activity.

Keywords: Antitumor activity, Basicity constant, Proton affinity, Schiff base

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Introduction

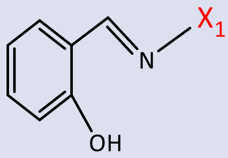
Compounds containing azomethine or imine group (R_2C=N-R′, R′≠H) are known as Schiff bases because they were first synthesized by Hugo Schiff. Schiff bases are synthesized by the reaction of aldehydes or ketones with primary amines under certain conditions [1,2]. Schiff bases are widely used ligands in coordination chemistry. When the general formula is examined, it is seen that there is a lone pair on the imine or azomethine nitrogen. Schiff bases can donate this lone pair to a Lewis acid and act as bases or monodentate ligands. In the case of a second deprotonizable functional group close to the azomethine group, Schiff bases act as a bidentate ligand forming complex compounds. In 2-((phenylimino)methyl)phenol, the phenol OH group is close to the azomethine group and is a deprotonizable group. Therefore, the 2-((phenylimino)methyl)phenol anion behaves as a bidentate ligand with N and O donor atoms [3-5].

Schiff bases and their metal complexes have an important place in the field of health due to their biological, pharmacological, antitumor properties and chelating abilities [6-9]. Schiff bases are the most widely used compounds as pigments, dyes, catalysts and polymer stabilizers. In addition, Schiff bases have been reported to exhibit a wide range of biological activities such as antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic [10,11]. Imine or azomethine groups are found in a variety of natural compounds, naturally derived compounds, and unnatural compounds. The imine or azomethine groups in such compounds are of critical importance for biological activity [12,13].

There is information in the literature that molecular descriptors such as hardness, softness, electronegativity, HOMO energy, LUMO energy, energy gap and electrophilicity index play a role in the chemical reactivity of Schiff bases [14-19]. The presence of electron donating groups in Schiff bases has been found to increase both antitumor and antibacterial activity [14,20]. In addition, it has been reported that the stability constants of Schiff base metal complexes are an important factor in their biological activity and that such complexes with high stability constants have high biological activity [21-23]. However, there is no information on whether there is a relationship between the basicity strength and biological activity of Schiff bases.

In this study, we aimed to calculate the molecular structures, basicity constants and proton affinities of 2-((phenylimino)methyl)phenol derivative Schiff bases and to investigate the effect of basicity strength on antitumor activity. In order for the basicity constants to fall into a wide range, we attached methy, ethyl, isopropyl and chloro groups to the imino on the 2-((phenylimino)methyl)phenol and designed Schiff bases, whose schematic structures and names are given in Scheme 1. Here, CH_3 is a weak electron donor group (EDG), Cl is a weak electron withdrawing group (EWG).

Schematic structure



X_1	Name
C_6H_5	(E)-2-((phenylimino)methyl)phenol (**HL1**)
CH_3	(E)-2-((methylimino)methyl)phenol (**HL2**)
$CH(CH_3)_2$	(E)-2-((isopropylimino)methyl)phenol (**HL3**)
Cl	(E)-2-((chloroimino)methyl)phenol ((**HL4**)
CH_2CH_3	(E)-2-((ethylimino)methyl)phenol (**HL5**)

Scheme 1. Schiff bases designed to calculate basicity constants and proton affinities.

The basicity constants and proton affinities of the Schiff bases given in Scheme 1 were calculated from the parameters obtained at the optimal level of B3LYP/6-31G(d). The same Schiff bases were docked against MCF-7 and A549 cell lines. Binding energies (BE), interaction energies (IE), and total interaction energies (TIE) were calculated. It was investigated whether there is a relationship between basicity constants and antitumor activity.

Methods and Techniques

The schematic structures of Schiff bases based on 2-((phenylimino)methyl)phenol were drawn in the program GaussView 6.0.16 [24]. Calculations were made in Gaussian 16, Revision C.01 program [25]. The basicity constants of HL1, HL2, HL3, HL4 and HL5 Schiff bases were calculated at twelve different levels, benchmark analysis was performed. No negative frequency was observed in any of the calculations made in the gas and aqueous phase at the optimal level [26]. B3LYP is one of the most widely used hybrid density functional theory methods. This method matches the Becke-style three-parameter exchange functional with the correlation functional of Lee, Yang and Parr [27]. 6-31G(d) is a polarized basis set that adds d functions to heavy atoms, commonly used to calculate medium-sized systems [28]. The molecular structures of HLn Schiff bases were characterized by calculating the lengths of some characteristic bonds, bond angles, IR stretching frequencies and NMR chemical shift values of some atoms. NMR spectra of some atoms were calculated by the GIAO method. Chemical shift values were obtained from the equation (1) [26].

$$\delta = \sum TMS - \sum \quad (1)$$

Here, ΣTMS is the shielding of carbon or hydrogen in TMS, Σ is the shielding of carbon or hydrogen in the sample, and δ is the chemical shift of carbon or hydrogen.

According to the molecular orbital theory, since the acidity of the molecules depends on the LUMO energy and the basicity depends on the HOMO energy, the E(HOMO) and E(LUMO) values of the Schiff bases were taken from the calculation output file. Some molecular descriptors such as ionization energy (I), electron affinity (A), energy gap (ΔE), hardness (η), Mulliken electronegativity (χ) and electrophilicity index (ω), which depend on HOMO and LUMO energy, were calculated from the following equations [14].

$$\delta = \sum TMS - \sum \quad (1)$$

$$I = -E_{HOMO} \quad (2)$$

$$A = -E_{LUMO} \quad (3)$$

$$\Delta E = E_{LUMO} - E_{HOMO} \quad (4)$$

$$\eta = \frac{I - A}{2} \quad (5)$$

$$\chi = \frac{I + A}{2} \quad (6)$$

$$\omega = \frac{\chi^2}{2\eta} \quad (7)$$

Relationships between calculated molecular descriptors and basicity constants were investigated. Since the basicity constants of Schiff bases are calculated in the aqueous phase, the aqueous phase calculations were made with the conductor-like polarizable continuum (C-PCM) model [29]. In order to determine the basicity constants (Kb) of the Schiff bases given in Scheme 1, the dissociation reaction of the proton from the imine nitrogen was taken into account.

$$H_2L^+(s) \rightleftharpoons HL(s) + H^+(s) \tag{8}$$

The more strongly the imine nitrogen binds to the proton in the $H2L^+$ ion, the lower the acidity and the higher the basicity. The standard Gibbs free energy change (∆Gs) of this reaction in solution is given by equation (9) [30].

$$\Delta G_s = G_s(HL) + G_s(H^+) - G_s(H_2L^+) \tag{9}$$

Here, Gs is the sum of the electronic energy (EE) and the thermal free energy correction term for each species, and it is taken directly from the output file. The $G(H^+)$ for the proton was taken from the literature [31]. $Gg(H^+)$=-6.29 kcal/mol for gas phase and $Gs(H^+)$=-263.977 kcal/mol for solution phase. There is a relationship given by equation (10) between the Gibbs free energy change and the acidity constant (Ka).

$$\Delta G_s = -RT\ln K_a \tag{10}$$

If this equation is arranged according to pK_a, the equation (11) is obtained [32].

$$pK_a = \frac{\Delta G_s}{2.303RT} \tag{11}$$

When ∆Gs is determined by computational methods, pK_a values can be calculated from this equation. Since $H2L^+$ and HL are conjugate acid base pairs, there is a relationship between pK_a and pK_b given by equation (12).

$$pK_a + pK_b = 14 \tag{12}$$

The proton affinity (PA) of Schiff bases is a measure of the basicity strength in the gas phase. To determine the proton affinity of Schiff bases, Schiff bases were protonated from the imine nitrogen according to equation (13). The gas phase energy of this reaction can be calculated from equation (14) [33].

$$HL(g) + H^+(g) \rightleftharpoons H_2L^+(g) \tag{13}$$

$$PA = E_g(H_2L^+) - E_g(HL) \tag{14}$$

Here, Eg is the sum of electronic energy (EE) and zero-point energy (ZPE). HLn Schiff bases, whose Kb and PA values were calculated, were docked to proteins with PDB codes 1M17 and 3HY3 in A549 and MCF-7 cell lines. Docking poses, interaction energies (IE), binding energies (BE), and total interaction energies (TIE) were calculated. IE, BE, and TIE plots were drawn against pK_b to investigate whether there is a relationship between the basicity constant and antitumor activity.

Findings and Discussion

Benchmark Analysis

In computational chemistry, there are many methods and many basis sets that can be used for a computational operation. In computational chemistry, a method/basis set combination is called level, and the process of determining the optimal level is called benchmark analysis. Benchmark analysis is done between a calculated feature at a certain level and an experimentally determined feature. The pKa values of HL1, HL2, HL3, HL4 and HL5 Schiff bases considered in this study were determined experimentally in a 60% ethanol + 40% water mixture solution [34]. For benchmark analysis, Schiff bases HL1, HL2, HL3, HL4 and HL5 and their protonated species from the imine nitrogen $H2L1^+$, $H2L2^+$ $H2L3^+$, $H2L4^+$ and $H2L^+$ were optimized at 12 different levels. These levels are HF/6-31G, HF/6-31G(d), HF/6-31G(d,p), HF/6-31++G, HF/6-31++G(d), HF /6-31++G(d,p), B3LYP/6-31G, B3LYP/6-31G(d), B3LYP/6-31G(d,p), B3LYP/6-31++G, B3LYP/ 6-31++G(d) and B3LYP/6-31++G(d,p). Standard Gibbs free energy change (∆Gs) of the proton dissociation reaction was calculated from equation (2) and pK_b values were calculated from equation (4). Calculated pK_b values of HL1, HL2, HL3, HL4 and HL5 Schiff bases in aqueous phase at 12 different levels are given in Table 1.

Table 1. The calculated for HL1-HL4 Schiff bases electronic energy (EE) and thermal free energy correction term (Hartree) and calculated pK_b values at the B3LYP/6-1G(d) level, protonated $H2L^+$ cations and L^- anions.

B3LYP/6-31G(d) ***Molecules (HLn)***	***H_2L^+***	***HL***	***L^-***	***pK_b***
HL1	-632,252425	-631,821043	-631,33517	9,08
HL2	-440,560951	-440,124324	-439,633815	6,66
HL3	-519,142594	-518,705382	-518,214780	6,39
HL4	-860,823954	-860,413141	-859,939372	18,54
HL5	-479,852210	-479,414653	-478,923668	6,23

When the phenyl group is removed from the structure and substituents are directly attached to the azomethine group, the pK_b values change significantly, either increasing or decreasing. In Schiff bases HL2, HL3, and

HL5, the attachment of alkyne groups to the azomethine moiety resulted in pK_b values ranging from 6.23 to 6.66. In contrast, HL11, the substitution of a more electronegative chlorine atom at the same position resulted in a markedly increased pK_b value of 18.54.

The pK_b values were calculated to determine the basic strength of Schiff bases HL1-HL5. As the pK_b value of a base decrease, its strength increases. In this study, the pK_b values of Schiff bases HL1, HL2, HL3, HL4, and HL5 were determined in aqueous solution.

Ground State Molecular Structures

Molecular properties depend on molecular structures. Therefore, it is important to identify the molecular structures. In computational chemistry, molecular structures can be determined by optimizing the molecule at the optimal level, comparing the characteristic bond lengths, bond angles, bond stretching frequencies, and chemical shift values of characteristic atoms with literature. In this study, ground state molecular structures of HLn (n=1-5) Schiff bases were optimized in the aqueous phase at the B3LYP/6-31G(d) level. Optimized structures are given in Figure 1.

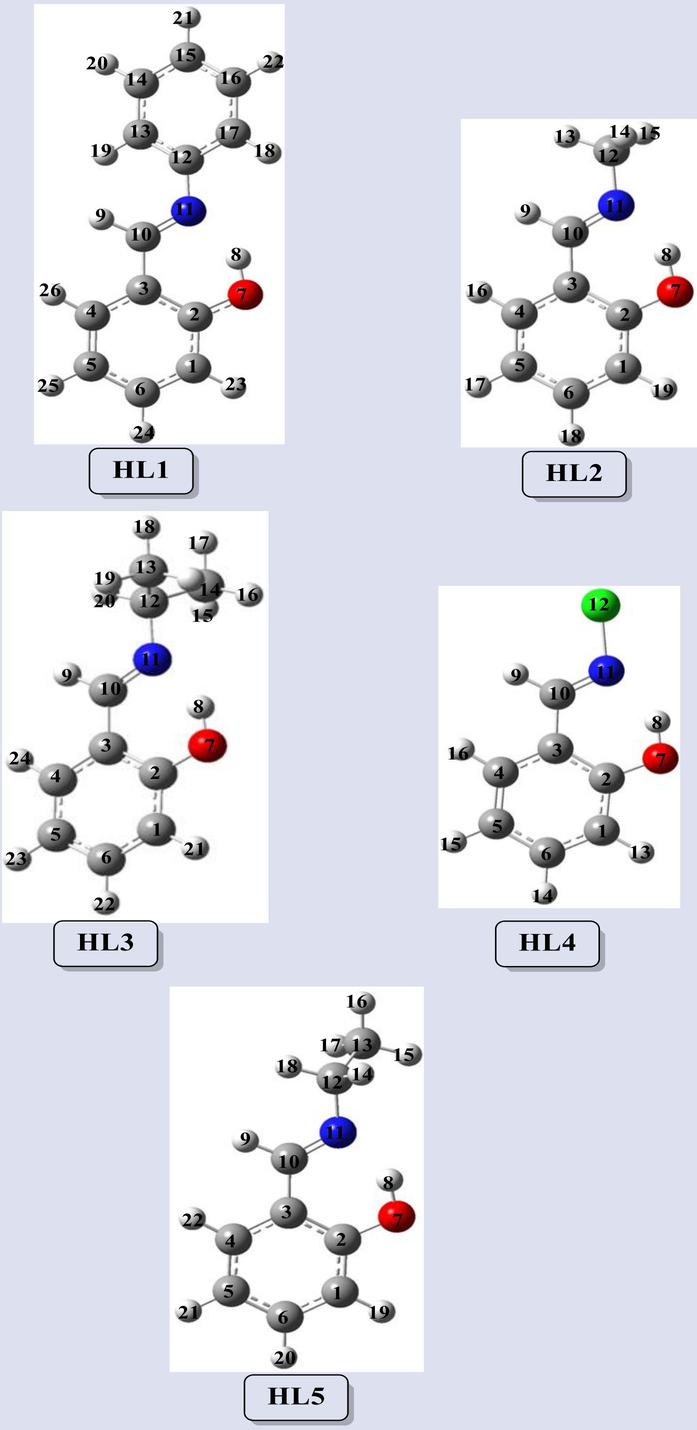


Figure 1. Ground state molecular structures of HL1-HL5 Schiff bases.

Stretching Frequencies of Characteristic Bonds

The characteristic C–O, C=N, C–H, and O–H bond stretching frequencies serve as important indicators for determining the molecular structures of 2-((phenylimino)methyl)phenol, traditionally known as salicylidene-aniline derivative Schiff bases. These stretching frequencies were calculated at the B3LYP/6-31G(d) level in the aqueous phase, and the harmonic frequencies are summarized in Table 2. As shown, in aqueous medium, the phenolic C–O stretching frequencies range from 1330 to 1376 cm^{-1}, azomethine C=N from 1655 to 1720 cm^{-1}, azomethine C–H from 3000 to 3130 cm^{-1}, and phenolic O–H from 3160 to 3365 cm^{-1}. For comparison, the IR spectrum of a similar molecule, salicylidene-2-hydroxyaniline, recorded in DMF solvent, shows C–O, C=N, and O–H stretching frequencies at 1274, 1631, and 3422 cm^{-1}, respectively [35]. Applying a scale factor of 0.9613 to the harmonic frequencies calculated at the B3LYP/6-31G(d) level converts them into anharmonic frequencies [36]. Multiplying the calculated harmonic frequencies by this factor shows good agreement between the computed and experimental values for the C–O and C=N stretching modes.

However, a notable discrepancy exists between the experimental O–H stretching frequency at 3422 cm^{-1} and the computationally predicted O–H stretching frequency for salicylidene-2-hydroxyaniline. This difference may arise from two factors: first, the solvent environment differs the experimental value was obtained in DMF, which exhibits little to no hydrogen bonding with the O–H group. In contrast, hydrogen bonding is likely between the O–H group of the salicylidene-aniline Schiff bases and surrounding water molecules in the aqueous phase, which can weaken the O–H bond and lower its stretching frequency. Second, the 3422 cm^{-1} band may correspond to the O–H group attached to the aniline moiety rather than that attached to the salicylidene portion. The calculated IR spectra for HL1–HL5 Schiff bases are presented in Figure 2.

Table 2. Calculated bond stretching frequencies (cm^{-1}) in aqueous medium for some characteristic bonds of Schiff bases

B3LYP/6-31G(d) Molecules (HLn)	ν(O-H)	ν(N=C-H)	ν(C=N)	ν(C=C)	ν(C-O)
HL1	3219,69	3061,71	1686,81	1537,22	1376,34
HL2	3163,13	3004,9	1720,2	1515,93	1337,44
HL3	3166,36	3023,9	1709,21	1513,03	1336,53
HL4	3365,24	3130,96	1657,51	1539,4	1330,23
HL5	3160,84	3024,34	1713,43	1518,28	1337,64

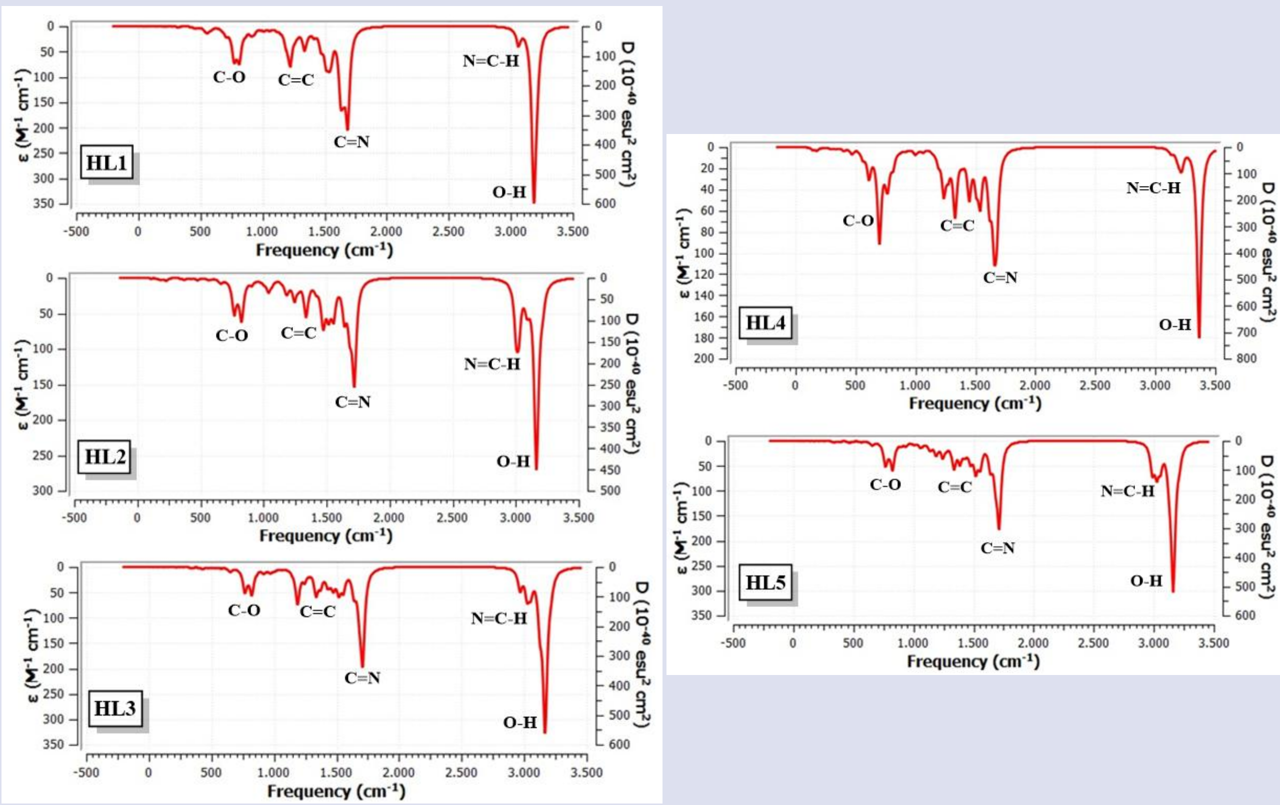


Figure 2. Gas-phase IR spectra of HL1–HL5 Schiff bases calculated at the B3LYP/6-31G(d) level.

Chemical Shift Values of Characteristic Atoms

One of the most used techniques to characterize the molecular structures of Schiff bases is to consider the chemical shift values of characteristic atoms in molecules. Therefore, ^{1}H-NMR and ^{13}C-NMR spectra of salicylidene-aniline derivative Schiff bases were calculated by Gauge-including atomic orbitals (GIAO) method at B3LYP/6-31G(d) level in aqueous phase. At the same level, shielding of TMS protons and carbons in aqueous medium was found to be 32.18 ppm and 189.71 ppm, respectively. The chemical shift values (δ) of some atoms thought to be characteristic for Schiff bases were calculated from equation (1) and given in Table 3.

Table 3. ^{1}H-NMR and ^{13}C-NMR chemical shift values (ppm) calculated for some characteristic atoms of Schiff bases in aqueous medium

	^{1}H-NMR		^{13}C-NMR	
Molecules (HLn)	**δ(*H*C10=N)**	**δ(O-*H*)**	**δ(*C*-O)**	**δ(*C10*=*N*)**
HL1	8.42	11.57	154.2	156.5
HL2	8.16	11.27	153.6	160.1
HL3	8.34	11.33	153.6	156.8
HL4	8.47	9.14	152.6	169.8
HL5	8.24	11.37	153.7	158.5

As seen from the ^{1}H-NMR chemical shift values in Table 3, the chemical shift values of the proton bound to the azomethine carbon vary in the range of 8.2-8.8 ppm, and the chemical shift values of the phenolic O-H proton vary in the range of 10-12 ppm. For similar molecules in the literature, the chemical shift values of the proton attached to the azomethine carbon vary between 8.40 and 8.6 [37] and the chemical shift values of the phenolic O-H proton vary between 11-13 ppm [14]. These values appear to be in good agreement. When the ^{13}C-NMR chemical shift values are examined, the chemical shift values of the carbon atom to which the OH group is attached are around 154 ppm and the chemical shift values of the azomethine carbon are between 156-168 ppm. For similar Schiff bases in the literature, the chemical shift values of the carbon atom to which the OH group is attached are 145-154 ppm [14] and the chemical shift values of the azomethine carbon are around 156 ppm [38].

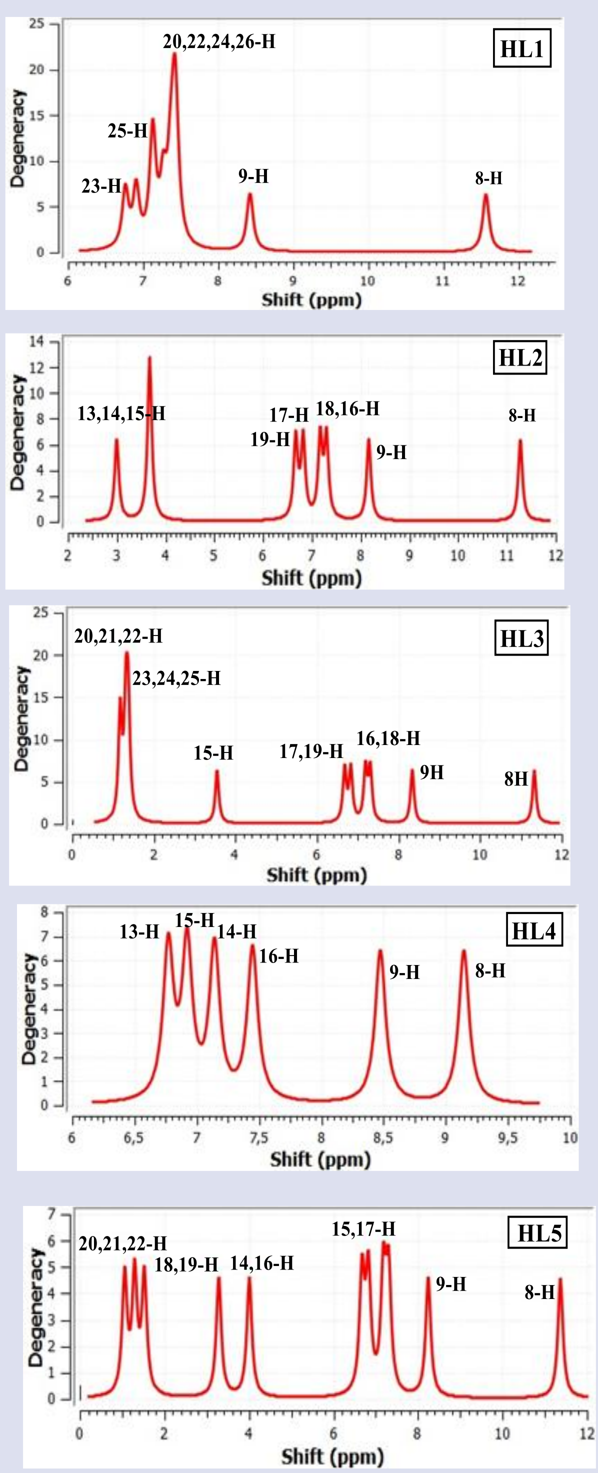


Figure 3. ^{1}H-NMR spectra of HLn Schiff bases calculated in aqueous phase using the GIAO method at the B3LYP/6-31G(d) level.

These results show that the calculated ^{13}C-NMR chemical shift values are in good agreement with the values given in the literature. The calculated ^{1}H-NMR and ^{13}C-NMR spectra of HL1-HL5 Schiff bases are given in Figure 3 and Figure 4.

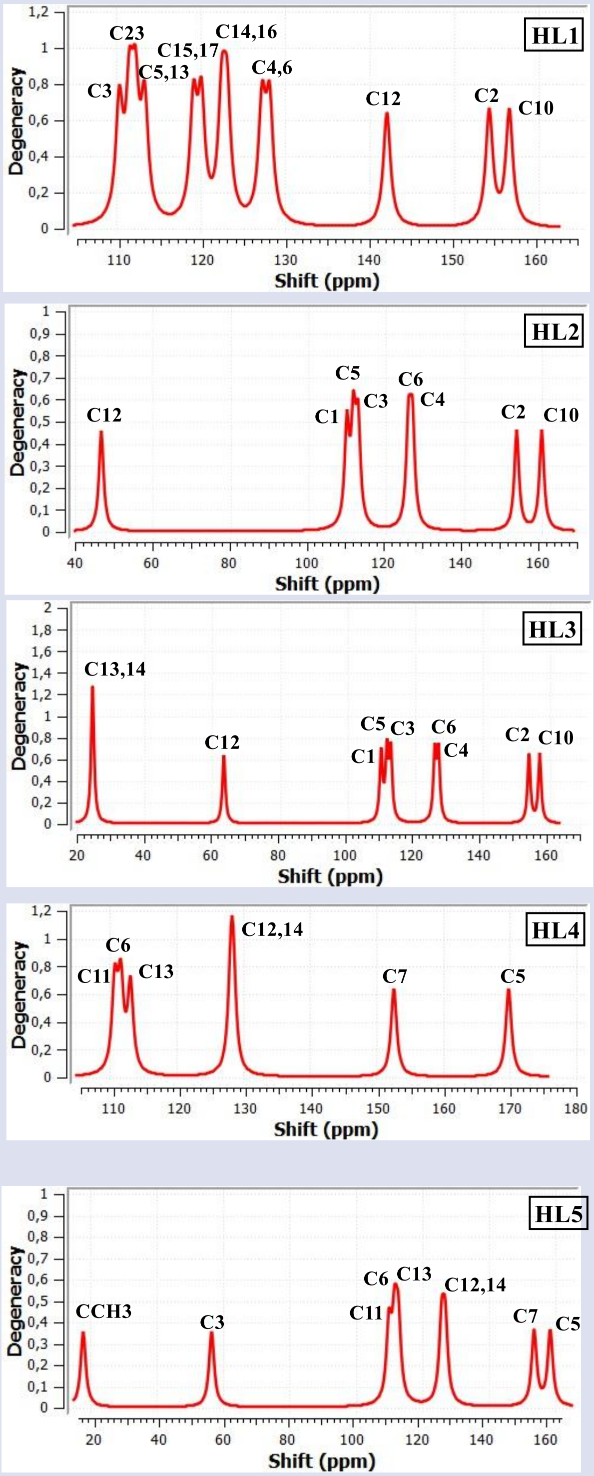


Figure 4. ^{13}C-NMR spectra of HLn Schiff bases calculated in aqueous phase using the GIAO method at the B3LYP/6-31G(d) level.

Basicity Strength

In computational chemistry, the highest occupied molecular orbital energy (EHOMO), basicity constant (pK_b), and proton affinity (PA) values can be considered to estimate the basicity strengths of molecules. Therefore, these parameters were calculated in this study.

HOMO energy

According to the molecular orbital theory, the acidity of the molecules depends on the LUMO energy and the basicity depends on the HOMO energy. Molecules with the lower LUMO energy behave more acidic and molecules with the higher HOMO energy behave more basic [39]. As seen from Equation (2)-(7), electronic structure descriptors such as ionization energy (I), electron affinity (A), energy gap (ΔE), hardness (η), Mulliken electronegativity (χ) and electrophilicity index (ω) depend on the HOMO and LUMO energies. Therefore, the molecular descriptors of Schiff bases considered in this study, depending on HOMO and LUMO energies, were calculated from equation (2)-(7) and given in Table 4.

Table 4. The energies of the frontier orbitals of HL1-HL5 Schiff bases and their molecular descriptors (eV)

Molecules (HLn)	E_{HOMO}	E_{LUMO}	I	A	ΔE	η	χ	ω
HL1	-5.955	-1.792	5.955	1.792	4.163	2.081	3.873	3.605
HL2	-6,021	-1,300	6,021	1,300	4,721	2,361	2,361	2,143
HL3	-6,016	-1,297	6,016	1,297	4,719	2,360	2,360	2,147
HL4	-6,309	-1,777	6,309	1,777	4,532	2,266	2,266	1,445
HL5	-6,020	-1,307	6,020	1,307	4,713	2,357	2,357	2,125

According to the molecular orbital theory, since molecules with higher HOMO energies will be more basic, the basicity order of Schiff bases examined according to their HOMO energies should be as follows.

HL4<HL2<HL5<HL3<HL1

According to this order, the weakest base is HL4 and the strongest base is HL3. This ranking is consistent with the order expected from the inductive effect of the methy, ethyl, isopropyl and chloro substituents. As seen in Figure 1, there are weak electron donating isopropyl, ethyl and methy in schiff bases are HL2, HL3 and HL5, and weak electron withdrawing Cl group in HL4, respectively. According to the inductive effect of these groups, weak electron donor CH_3 is expected to increase the electron density on the imine nitrogen and increase the basicity strength, while weak electron withdrawing Cl is expected to decrease the electron density on the imine nitrogen and decrease the basicity strength. The results expected from the inductive effects of the substituents are in agreement with the basicity order according to the HOMO energies. According to the inductive effect, increasing the number of electrons withdrawing groups is expected to decrease the basicity.

Basicity constants

The basicity constant is a quantitative measure of the basicity strength of bases in aqueous solution. The basicity constants of bases can be determined experimentally or computationally. In this study, the basicity constants of Schiff bases derived from salicylidene-aniline or systematically named 2-((phenylimino)methyl)phenol were determined computationally. For this process, five salicylidene-aniline derivative Schiff bases given in Scheme 1 were designed. To calculate the basicity constants, the dissociation reaction of the proton from the imine nitrogen was considered. HLn Schiff bases and their H_2Ln^+ type conjugate acids were optimized in the aqueous phase at the B3LYP/6-1G(d) level and Gibbs free energies were calculated. The Gibbs free energy of the H^+ ion in aqueous solution was taken from the literature as -263,977 kcal/mol. The pK_b values were obtained from Equation (8)-(12). Gibbs free energies of HLn Schiff bases and their H_2Ln^+ type conjugate acids and pKb values of Schiff bases are given in Table 5.

Table 5. Gibbs free energies and pK_b values of HLn Schiff bases and their H_2Ln^+ type conjugate acids

Molecules	Gs*(HL)	Gs*(H_2L^+)	pK_b
HL1	-396473.707	-396744.403	9.08 (9.66)
HL2	-276182.194	-276456.182	6.66
HL3	-325492.555	-325766.910	6.39
HL4	-539917.420	-540175.209	18.54
HL5	-300837.25	-301111.820	6.23

*The energies are in kcal/mol. In brackets are experimental values.

In the literature, pKa values for salicylideneaniline (HL1) is given as 4.34 [34]. According to these pK_a values, the pK_b values of Schiff bases is 9.66. As seen in Table 6, the pK_b values calculated in this study were 9.08 for HL1. These results show that the pK_b values calculated in this study are in good agreement with the values given in literature. Small differences between experimental and computational pK_b values may be due to solvent differences. Experimental pK_b values were obtained in 60% ethanol+40% water solvent, and computational pK_b values were obtained in 100% water solvent.

As seen in Table 5, the pK_b values of the designed Schiff bases range from 9 to 18. As the basicity increases, the Kb value increases while the pK_b value decreases. The smaller the pK_b value, the stronger the base. According to the calculated pK_b values, the basicity order of the investigated Schiff bases is as follows. Graph of E(HOMO) versus pK_b values of Schiff bases are given in Figure 5.

HL4<HL1<HL2<HL3<HL5

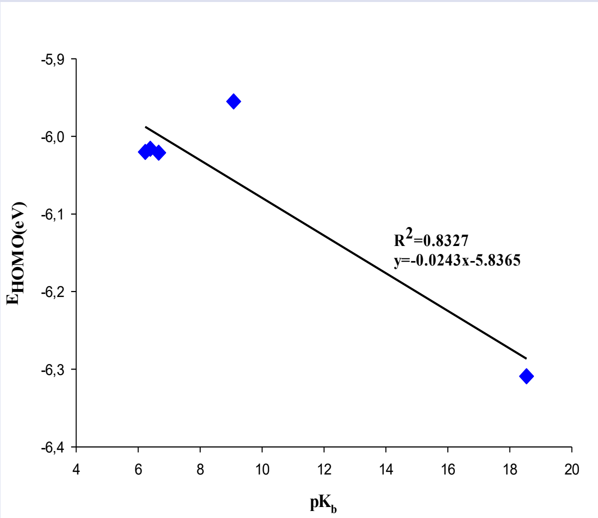


Figure 5. Graph of E(HOMO) versus pK_b values of Schiff bases.

Proton affinities

The proton affinity is taken as a quantitative measure of the strength of inherent basicity in the gas phase of the chemical species themselves. The proton affinity of a base is defined as the enthalpy of its reaction with H^+ in the gas phase. As the proton affinity of a chemical species increases, the basicity strength increases and the acidity strength of its conjugate acid decreases. In order to determine the proton affinity of HLn Schiff bases considered in this study, Schiff bases were protonated from the imine nitrogen according to equation (13) and their conjugate acids H_2Ln^+ were obtained. HLn Schiff bases and their H_2Ln^+ type conjugate acids were optimized in the gas phase at the level of B3LYP/6-31G(d). The total energies (E_g) in the gas phase were found for each species and the proton affinity (PA) was calculated from equation (14). The total energies (E_g) of HLn Schiff bases and their H_2Ln^+ cations in the gas phase and the proton affinities (PA) of Schiff bases are given in Table 6.

Table 6. Total energies (E_g) of HLn Schiff bases, H_2Ln^+ cations and proton affinities (PA) of Schiff bases

Molecules	Eg*(HL)	Eg*(H_2L^+)	-PA*
HL1	-631.775454	-632.144662	969.35
HL2	-440.085189	-440.525832	1156.92
HL3	-518.662080	-519.034846	978.70
HL4	-860.373548	-860.714650	895.57
HL5	-479.373222	-479.744273	974,20

*Total energies (Eg) are Hartree and proton affinities (PA) are kJ/mol.

As can be seen from Table 6, the proton affinity values of Schiff bases vary in the range of 895-1157 kJ/mol. When the experimental proton affinities of the amine derivatives given in the literature are examined, it is seen that it is in the range of 899-948 kJ/mol [33]. These results indicate that many of the investigated Schiff bases exhibit stronger basicity compared to the amine derivatives reported in the literature. The order of basicity strength in gas phase of Schiff bases investigated according to calculated proton affinity values is as follows.

HL4<HL1<HL5<HL3<HL2

This order is almost identical to the order expected from the inductive effects of the Schiff base substituents for salicylideneaniline (HL1).

When H attached to the ortho position of the azomethine group in salicylideneaniline (HL1) is referenced, CH3 is the weak electron donor, Cl is the weak electron withdrawing group.

As shown in Figure 1, in HL2, HL3, and HL5, the nitrogen atom is directly bonded to a linear electron-donating CH_3 group, which increases the electron density on the nitrogen atom. Consequently, the proton affinity and, therefore, the basicity of HL2, HL3, and HL5, increase accordingly. Graph of proton affinity (PA) versus pK_b values of Schiff bases are given in Figure 6.

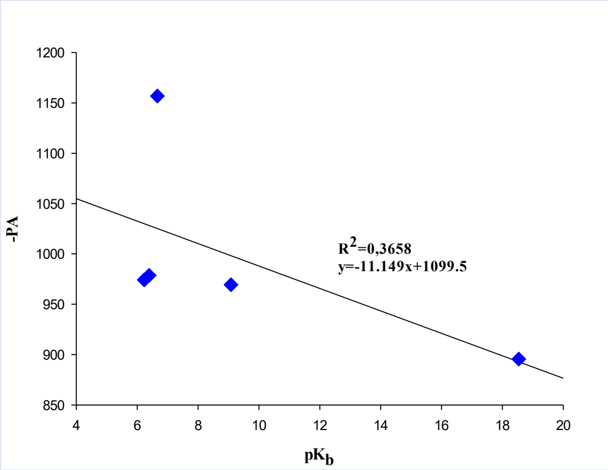


Figure 6. Graph of proton affinity (PA) versus pKb values of Schiff bases.

Docking against 3HY3 and 1M17 Proteins in MCF-7 and A549 Cell Lines

Molecular docking is a widely used method to predict the binding modes and binding energies of ligands to proteins. With this method, drug design can be made by calculating the energies of secondary chemical interactions between proteins and ligands. In this study, molecular docking was used to investigate whether there is a relationship between basicity strength and antitumor activity of HL1-HL5 salicylidene-aniline derivative Schiff bases, which are known to exhibit antitumor activity. Therefore, HL1-HL5 Schiff bases, whose basicity constant was calculated, were first docked against 5,10-methenyltetrahydrofolate synthetase (MTHFS) (PDB ID:3HY3) protein, which is known to be expressed in breast cancer cell line (MCF-7). Because inhibition of MTHFS in human MCF-7 breast cancer cells has been shown to stop the growth and proliferation of cells [40]. Schiff bases were then docked against the epidermal growth factor receptor kinase (EGFRK) (PDB ID: 1M17) protein, which is known to be expressed in the lung cancer cell line (A549) [41]. Ligand-protein docking poses, interaction types and various docking energies were calculated.

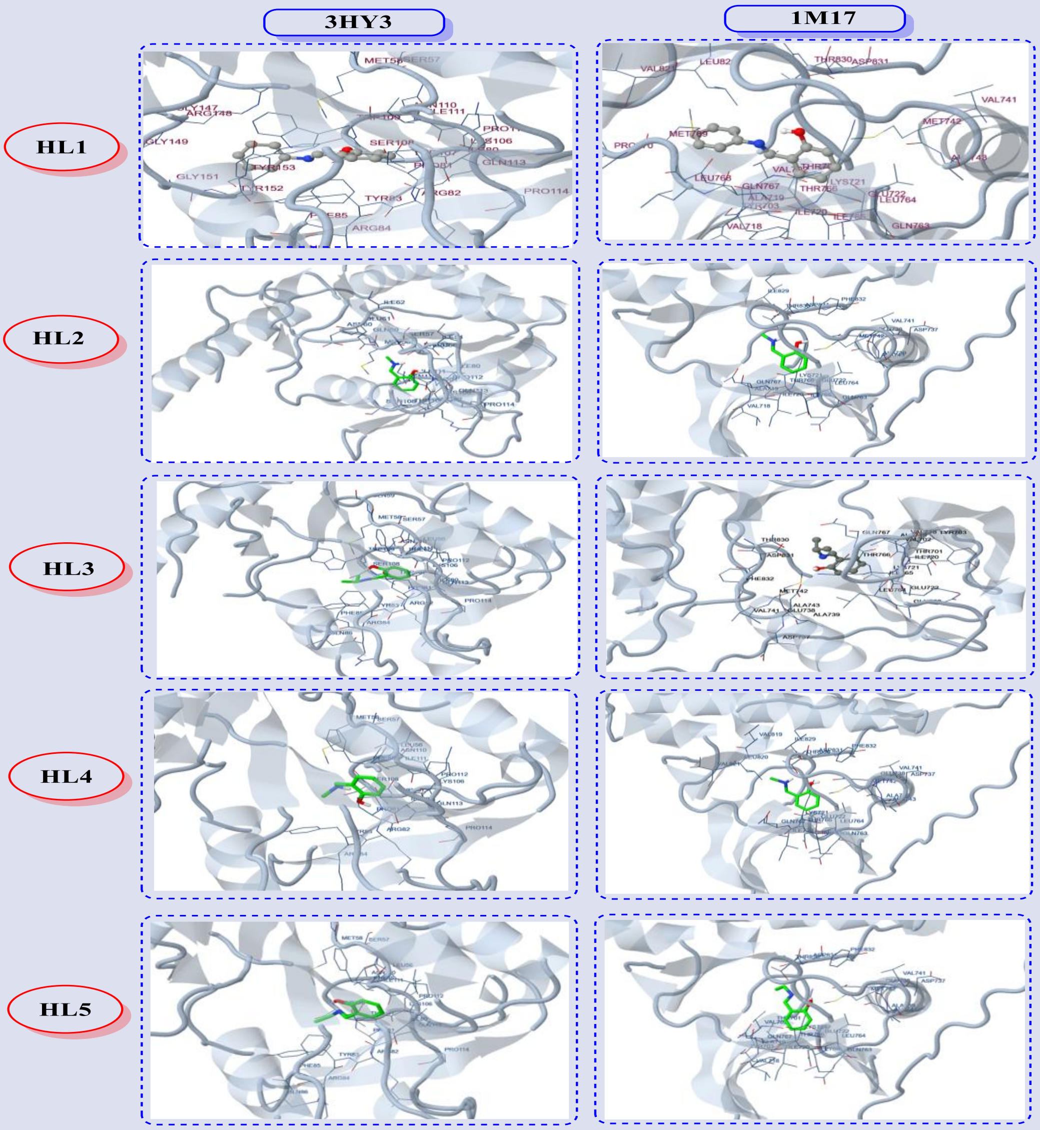


Figure 7. Docking poses between HL1-HL5 Schiff bases and 3HY3 and 1M17 proteins.

Molecular docking was done with the Docking Server program [43]. The optimized structures of HL1-HL5 ligands obtained at the B3LYP/6-31G(d) level in aqueous solution were docked into 3HY3 and 1M17 proteins whose crystal structures were taken from the RSCB protein data bank. Geometry optimization of ligand-protein complexes was done again on the docking server with the MMFF94 method. Gasteiger partial load calculation method was chosen. pH=7.0 was taken. Lamarckian genetic algorithm was used for insertion simulations. The 5A quaternion and torsion steps were applied to search for the appropriate region of the target protein. The docking poses of HL1-HL5 Schiff bases between the 3HY3 and 1M17 proteins are given in Figure 7 and the ligand-protein interaction types are given in Table 7.

Table 7. Types of interactions between HL1-HL5 Schiff bases and 3HY3 and 1M17 proteins

	3HY3				1M17		
	H- bonds	Polar	Hydrophobic	pi-pi	H- bonds	Polar	Hydrophobic
HL1	THR107	-	PRO81 ILE111	TYR83 PHE85 TRP109 TYR152	-	THR766 THR830	ALA719 MET742 LEU764 LEU820
HL2	LEU56	-	ILE111 PRO81 MET58	PHE55	-	LYS721 ASP831 GLU738 THR830	LEU764 MET742
HL3	-	-	TYR83 PRO81 LEU56 ILE111 TRP109	-	-	LYS721 GLU738	LEU764 VAL702 MET742
HL4	-	GLN113	PHE85 PRO81 LEU56 ILE111 MET58	TYR83	LYS721	THR830 GLU738	LEU764 MET742
HL5		THR107	PRO81 LEU56 TYR83 ILE111 PHE85	-	-	LYS721 GLU738	LEU764 VAL702 ALA719

The number and strength of secondary chemical interactions play an active role in docking parameters such as ligand-protein interaction energy and binding energy. As presented in Table 8, HLn Schiff bases predominantly engage in π-π, hydrophobic, and polar interactions with the 3HY3 protein, whereas their interactions with the 1M17 protein are mainly hydrophobic in nature. These results may indicate that Schiff bases will generally interact with the breast cancer cell (MCF-7) 3HY3 protein more strongly than the lung cancer cell (A549) 1M17 protein. This qualitative evaluation can be substantiated by analyzing the binding energy (BE), interaction energy (IE), and total intermolecular energy (TIE) values of the ligand-protein complexes. Therefore, BE, IE and TIE values of HLn Schiff bases were taken from the docking results and given in Table 8.

Table 8. Binding energy (BE), interaction energy (IE) and total intermolecular energy (TIE) values between HLn Schiff bases and 3HY3 and 1M17 encoded proteins

	3HY3-MCF-7			1M17-A549		
HLn	BE*	IE*	TIE*	BE*	IE*	TIE*
HL1	-6.42	-6.63	-6.70	-5.56	-5.88	-5.87
HL2	-3.83	-3.93	-4.54	-3.78	-3.67	-4.26
HL3	-5.50	-5.79	-5.76	-5.00	-5.23	-5.26
HL4	-5.27	-5.44	-5.55	-4.38	-4.48	-4.68
HL5	-5.04	-5.30	-5.28	-4.58	-4.81	-4.88

*kcal/mol

As seen in Table 8, BE, IE and TIE values between HLn Schiff bases and 3HY3 protein are greater in absolute value than the values between 1M17 protein. This result may show that Schiff bases interact more strongly with 3HY3 protein than 1M17 protein and Schiff bases are more active against breast cancer cells. This finding is also consistent with the number of secondary chemical interactions between the ligand and the protein.

Basicity Strength-antitumor Activity Relationship

The main purpose of this study is to calculate the basicity strength of Schiff bases and to investigate whether there is a linear relationship between basicity strength and antitumor activity. Therefore, E(HOMO), PA and pK_b values of HLn Schiff bases were calculated in the previous sections. By docking HLn Schiff bases to 3HY3 and 1M17 proteins, docking energies such as binding energy (BE), interaction energy (IE) and total intermolecular energy (TIE) were calculated. In order to investigate whether there is a linear relationship between basicity strength and anticancer activity, BE, IE and TIE values between 3HY3 and 1M17 proteins and Schiff bases were plotted against pK_b values. pK_b-(BE, IE, TIE) plots are given in Figure 8 and 9.

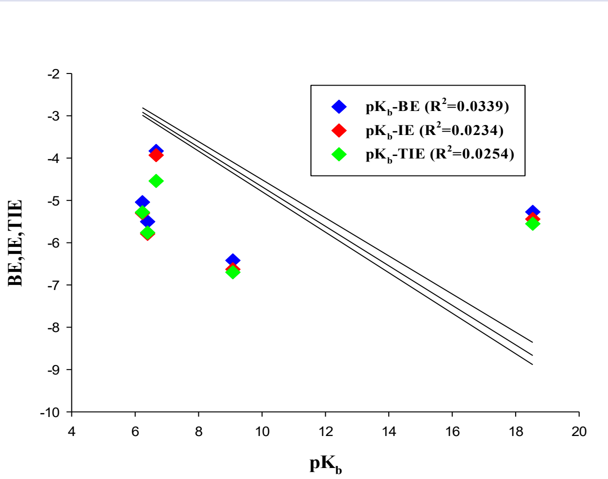


Figure 8. Graph of BE, IE and TIE values of 3HY3 protein versus pK_b values of HLn Schiff bases.

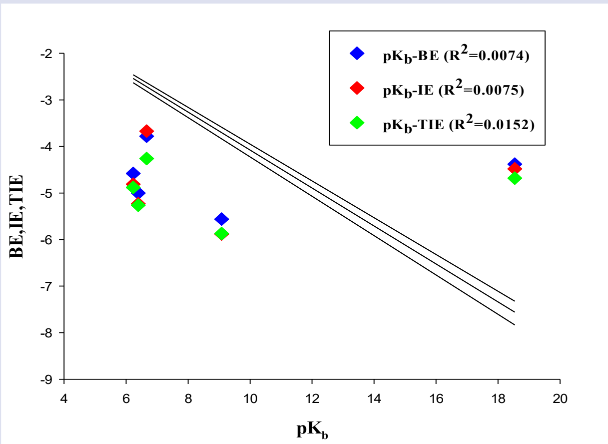


Figure 9. Graph of BE, IE and TIE values of 1M17 protein versus pK_b values of HLn Schiff bases.

As can be seen from Figure 8 and Figure 9, as the pK_b values of Schiff bases increase, the BE, IE and TIE values of both 3HY3 and 1M17 proteins decrease. More negative BE, IE, and TIE values indicate stronger ligand-protein interaction. In general, then, it can be said that as the pK_b value of Schiff bases increases (as the base strength decreases), the antitumor activity will be higher. However, the R^2 values given on the graphs show that this relationship is not linear. Therefore, a linear relationship between basicity strength and antitumor activity cannot be established. As seen from the E(HOMO), PA and pK_b values, Schiff bases are generally very weak bases. Perhaps the fact that Schiff bases are very weak bases causes them to have antitumor activity. In Figure 8 and Figure 9, it was found that there was an almost linear and inversely proportional relationship between the pK_b values of Schiff bases and the E(HOMO) and PA values.

The ligand-protein interaction is actually a secondary chemical interaction. In secondary chemical interactions, there is no change in the electronic structure of the substance. Therefore, only interactions such as hydrogen bonds, halogen bonds, polar interactions, hydrophobic or pi-pi interactions occur in the ligand-protein interaction. There is no chemical interaction such as acid-base interaction between Schiff bases and proteins. Therefore, a linear relationship between the basicity strength of Schiff bases and antitumor activity cannot be established. In chemical events, the electronic structure of matter changes. The change of electronic structure causes the formation of a new chemical species. This is not a desirable situation in drug design.

Conclusions

In this study, HL1-HL5 Schiff bases were designed by attaching methy, ethyl, isopropyl and chloro groups to the imino on the 2-((phenylimino)methyl)phenol Schiff base. The B3LYP/6-31G(d) level was determined as the optimal level. The ground state molecular structures of Schiff bases were characterized from Infrared stretching frequencies, ^{1}H-NMR and ^{13}C-NMR chemical shift values for HL1-HL5 Schiff bases. Some molecular descriptors and basicity constants of Schiff bases from aqueous phase optimization and proton affinities from gas phase optimization were calculated. It was found that there is an almost linear and inversely proportional relationship between HOMO energies and proton affinities and pK_b values. Schiff bases were docked to protein chains with PDB codes 3HY3 and 1M17, and their docking poses, binding energies (BE), interaction energies (IE) and total interaction energies (TIE) were calculated. It was found that the antitumor activity of Schiff bases with high pKb values was generally high. However, it was determined that there was no linear relationship between pKb value and antitumor activity.

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