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Synthesis, Characterization and Analytical Studies of Three Newly Schiff Bases As A New Anti-Human Breast Cancer (MCF-7)

Mustafa M. Grafa[a]*, Hanan M. Ali[b]

[a,b] *Education Directorate of Basrah, Ministry of Education, Basrah – Iraq*

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ABSTRACT

In this study, 2,5-dimethyl-4-((4-nitrobenzylidene) amino) phenol (A_1), *N,N'*-([1,1'-biphenyl]-4,4'-diyl)*bis*(1-(2,4-dimethoxyphenyl)methanimine (A_2) and 4,4'-(([1,1'-biphenyl]-4,4'-diyl*bis*(azaneylylidene))*bis* (methaneylylidene))*bis*(2-methoxyphenol) (A_3) were synthesised. Analytical studies were then carried on (A_1), (A_2) and (A_3); the results of the solvent effect were displayed that the best solubility was in ethanol and DMSO of each, which is due to the fact that the effect of the dielectric constant is the main factor that can control the shift of the absorption beaks. The results were also indicated, that there is a slight deviation from the linear relationship in (A_3) that may have related to the hydrogen bond between this base and the solvent. However, the results of the pH effect of (A_1), (A_2) and (A_3) in a range of buffer solution were gave two isopiestic points just in (A_3). Due to the ionization constant (pKa) and the protonation constant (pKp) were calculated by using the half height method. Further, the results were indicated the existence of the equilibrium schemes of which displays the suggested ionization of (A_3) in acidic and basic medium. Further, the effect of the prepared Schiff bases (A_1, A_2, and A_3) were studied on breast cancer cells type MCF-7 using five different concentrations of each. The results were showed high inhibition activities of each especially at high concentration, due recommend these bases as novel anti-cancer drugs.

1. Introduction

Schiff bases have been extensively studied, as they possess many interesting features, including photochromic and thermochromic properties, proton transfer tautomeric equilibria, biological and pharmacological activities, as well as suitability for analytical applications (Galić, et al., 2008). Due to synthetic flexibility and simple preparation procedure these compounds have received a great deal of attention as suitable ligands for coordination and determination of various metal ions. Many Schiff base metal complexes with a variety of biological activities have been described in the literature. Schiff bases are attractive as analytical reagents because they enable simple and inexpensive determinations of various organic and inorganic substances (Cimerman, et al., 1997). In general, there are two principal ways of their analytical application : first, determination of organic compounds bearing an amino or an active carbonyl group by the formation of coloured (chromophore-containing), fluorescent or insoluble Schiff bases, and secondly, the determination of various metal ions, as well as amino and carbonyl compounds, using complex formation reactions. The analytical methods based on complex formation are used more frequently. Owing to the relatively simple preparation procedures of schiff bases, it is possible to obtain ligands of different design and characteristics by selecting appropriate reactants. A phosphate buffer was found to significantly promote tetra-methyl pyrazine (TMP) formation in an acetoin (3-hydroxy-2- butanone)/ ammonium hydroxide system (Huang, 1997). The effect of the phosphate ion on TMP formation was additive in the range of 0.05-0.2 M.

***Corresponding author:** Mustafa M. Grafaa, Education Directorate of Basrah, Ministry of Education, Basrah – Iraq

E-mail: **mostafamohsen069@gmail.com**

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The change in pH value of the system reveals that a proton-coupled redox type of reaction occurred during TMP formation. Phosphate serves both as proton donor and acceptor to facilitate proton transfer during the Schiff base formation between ammonia and 3-hydroxy-2-butanone. Protic solvents, methanol and ethanol, were found to attract the water released from the system. The combination of a phosphate buffer and protic solvent led to the completion of TMP formation. The TMP formation mechanism in a phosphate buffer (pH 7.2) is proposed. The possibility of using a Schiff base as an acid-base indicator (Khalil, et al., 2009), this surprising phenomenon can be considered as an interest due to the fact that Schiff bases are usually unstable in solutions and definitely undergo hydrolysis. It was found that such a specific observation depends merely upon the chemical structure and type of the substitute of amine that reacts with aldehyde to give the Schiff base. The latter reagent4[(4-dimethylamino-benzylidene)-amino]-benzene sulfonamide was synthesized from the condensation of sulfanilamide with *p*-N-dimethyl amino benzaldehyde. The reagent solution shows a reproducible change in its color due to the addition of acid and base. A UV-Vis spectroscopic characterization and acid-base equilibrium study of the reagent for its possible use as an indicator were investigated.

The results show that the reagent is an amphoteric which possesses four ionization constants Ka1, Ka2, Kb1 and Kb2 of weak dibasic and diacidic properties. The value of pKa2 (9.80) is parallel to the observed transition interval pH 9.5 (yellow) and pH 10.5 (colorless), which is considered to be the indicator exponents pKi. It was concluded that the benzyl sulfonamide group plays a key role in the stability of the reagent towards hydrolysis and for indicator characteristics through breaking the conjugation. Breast cancer is a category of malignant tumors that arise from within the breast tissue and arise mainly in the milk ducts or connected lobules in the mammary gland. It affects both men and women, but the infection in men is rare (Parkin, et al., 2005).

There is currently no chemotherapy strategy for treating breast cancer. Therefore, finding new and better drugs is one of the priorities for the treatment of breast cancer (Majeed, et al., 2014). The limited effectiveness of common treatments for advanced breast cancer is an incentive for collaborative efforts to identify chemotherapy agents for effective treatment. This process often involves the use of metal complexes (Banerjee & Chakravarty, 2015). Cisplatin, which is recommended to nearly half of the cancer patients (Johnstone, et al., 2014; Zhang & Lippard, 2003), is still considered as one of the most important anticancer drugs. This drug and carboplatin as the second generation platinum drugs opened the way for screening a number of metal-based drugs for their anti-cancer activities (De Vizcaya-Ruiz, et al., 2000). In spite of the success of this drug, its clinical use is limited due to significant side effects (Hosseini, et al., 2017).

2. Materials and Methods

2.1. Synthesis of the Three Newly Schiff Bases

Each of 4-nitrobenzaldehyde (453 g, 0.003 mmole), 2, 4-*di* methoxybenzaldehyde (0.664 g, 0.004 mmole) and vanillin (0.608 gm 0.004 mmole) were stirred in separated round bottom flask and heated in each solution mixture of 4-amino-2, 5-dimethylphenol (411 g, 0.003 mmole), benzidine (0.368 g, 0.002 mmole) and benzidine (0.368 g, 0.002 mmole), that dissolved in 20 mL, 25 mL and 25 mL of absolute ethanol respectively and 3 drops of acetic acid was add to each with reflux. After 5 hours each mixture of (A_1), (A_2) and (A_3) was filtrated and each precipitate was recrystallized using ethanol and hexane to result a yellow, yellow and orange Schiff bases, that yield: (1.15 g, 85%), (1.20 g, 80%) and (1.30 g, 87%) respectively, m.p : (136-138), (252-254) and (230-232) C°. This showed δ H (500 MHz, DMSO): 2.5 (s,6H, DMSO), 3.3 (s, H, HOD), 2.2,2.4 (s, 6H, CH3), 6.5-8.5 (s, 6H, Ar-H), 8.7 (s,1H, CH=N), 9.4 (s, H, OH), (A_1); (Sondhi, et al., 2009), 2.5 (s,6H, DMSO), 3.3 (s, H, HOD), 3.8 (s, 12H, OCH3), 6.5 - 8 (m, 14H, Ar-H), 8.7 (s, 2H, CH=N), (A_2) and 2.5 (s, 6H, DMSO), 3.3 (s, H, HOD), 3.8 (s, 6H, OCH3), 6.8–7.8 (m, 14H, Ar-H), 8.5 (s, 2H, CH=N), 9.8 (s, 2H, OH), (A_3) (Coombs, et al., 2005).

2.2. Solution of Three Newly Schiff Bases in Ethanol

The solutions (50 mL) of each of (A_1), (A_2) and (A_3) in ($1*10^{-3}$ M) concentration.

2.3. The Solvent Effect

The solution of each of (A_1), (A_2) and (A_3) was prepared by dissolved in different solvents, (ethanol, methanol, DMSO and chloroform) to give ($1x10^{-4}$ M) concentration from each.

2.4. The pH Effect

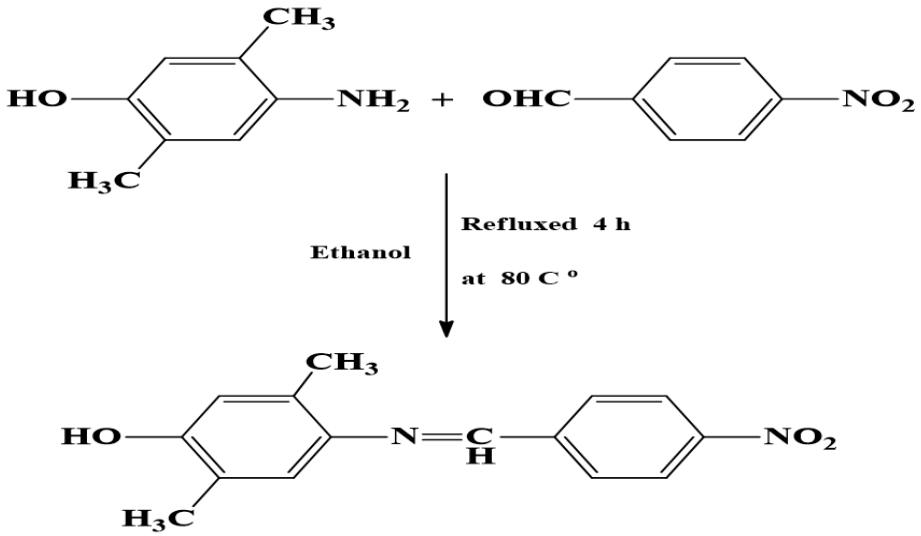
The solution of each of (A_1), (A_2) and (A_3) was prepared by dissolved in a range of different buffer solutions, (2-12) to give ($1x10^{-4}$ M) concentration from each.

2.5. Anticancer Study

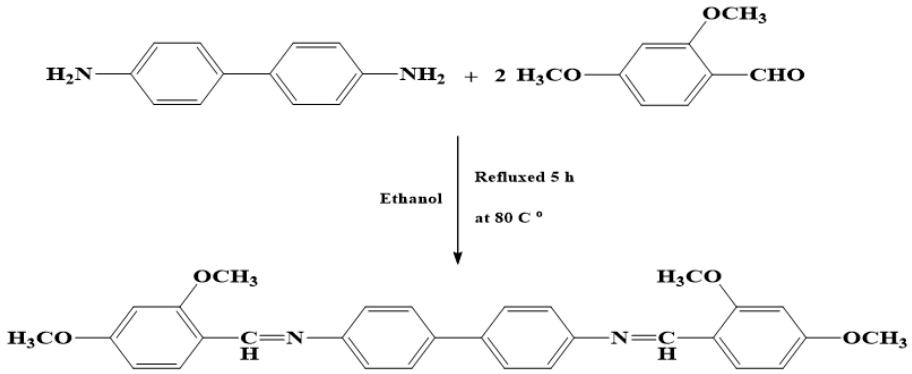
The cell growth and cell viability were quantified using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide] assay. In brief, for monolayer culture, cells (MCF7) were digested with trypsin, harvested, adjusted to a density of 1.4×10^4 cells/well, and seeded to 96-well plates filled with 200 µl fresh medium per well for 24 h. When cells formed a monolayer, they were treated with 500-15.62 µg/ml of the compounds for 24 h at 37 °C in 5% CO_2. At the end of the treatment (24 h), while the monolayer culture was left untouched in the original plate, the supernatant was removed, and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added, and the plate was incubated at 37 °C for an additional 4 h. MTT solution (the supernatant of cells was removed, and dimethyl sulfoxide was added (100 µl per well). Cells were incubated on a shaker at 37 °C until crystals were completely dissolved. Cell viability was quantified by measuring absorbance at 570 nm using an ELISA reader (Model wave xs2, BioTek, USA).

3. Results and Discussion

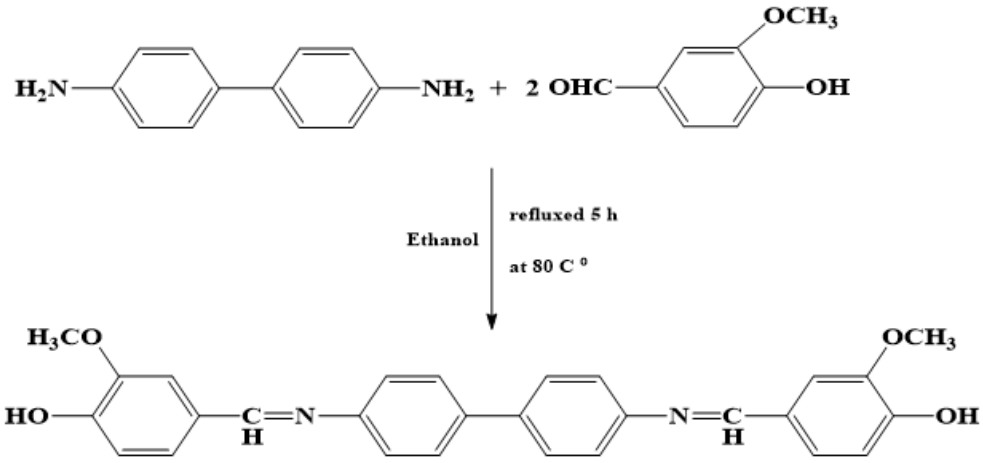
The 2,5-dimethyl-4-((4-nitrobenzylidene) amino) phenol (A_1), *N*, *N*'-([1,1'-biphenyl]-4,4'-diyl) *bis* (1-(2,4-dimethoxyphenyl) methanimine (A_2) and 4,4'-(([1,1'-biphenyl]-4,4'-diyl*bis*(azaneylylidene)) *bis* (methaneylylidene)) *bis* (2-methoxyphenol) (A_3) were synthesised, (Scheme 1, 2 and 3).



Scheme 1 The synthesis of Schiff base (A_1)



Scheme 2 The synthesis of schiff base (A_2)



Scheme 3 The synthesis of schiff base (A_3)

The synthetic schiff bases were characterized by IR spectrum, mass spectrum, ^{1}H NMR spectrum and UV-visible spectrum. The IR spectrum of the (A_1), and (A_3), (Figures 1 and 3) were showed the stretching vibration of the ν (O-H)

groups in the regions 3236 cm^{-1} and 3485 cm^{-1} respectively. But, the ν (CH=N) stretching vibration band of (A_1), (A_2) and (A_3) were appeared in the region 1622 cm^{-1}, 1604 cm^{-1} and 1622 cm^{-1} respectively (Silverstein & Bassler, 1962; Baluja, et al., 2006). Therefore, the mass spectrum was showed that the peak of (A_1), (A_2) and (A_3) at *m/z* were equal to 270, 480 and 452 as seen in Figures 4, 5 and 6 respectively. Add to which, the ^1H NMR spectrum was confirmed the formation of each of (A_1), (A_2) (A_3), (Figures 7, 8 and 9). The UV-visible spectrum was documented at the range (200-500) nm in ethanol for each of the synthetic Schiff bases, (Figures 10-12). The absorption spectrum of (A_1), (A_2) and (A_3) were showed bands at (270 nm and 310 nm); (275 nm and 305 nm) and (280 nm) respectively related to (π -π*) (Fasina, et al., 2013).

Analytical studies were also curried on (A_1), (A_2) and (A_3). First, the solvent effect of each was studied, (Figures 10-12) using set of different solvents, (chloroform, methanol, ethanol and DMSO). The results were showed that the best solubility of (A_1) was in DMSO, but the best solubility of schiff bases (A_2) and (A_3) was in ethanol.

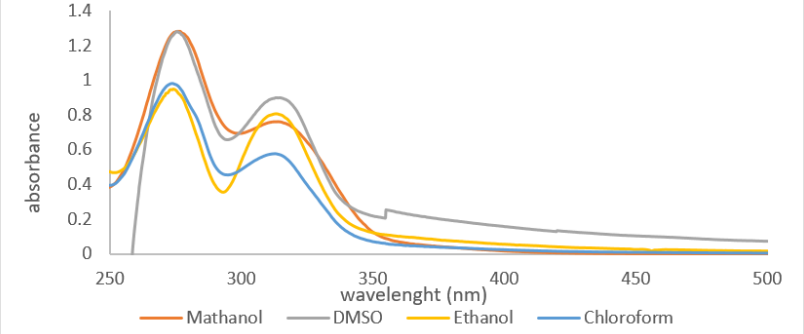


Fig. 1. Electron absorption spectra of Schiff base (A_1) in different organic solvents

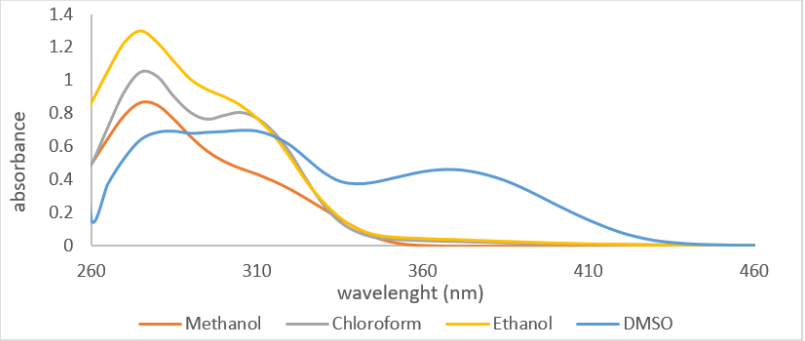


Fig. 2. Electron absorption spectra of Schiff base (A_2) in different organic solvents

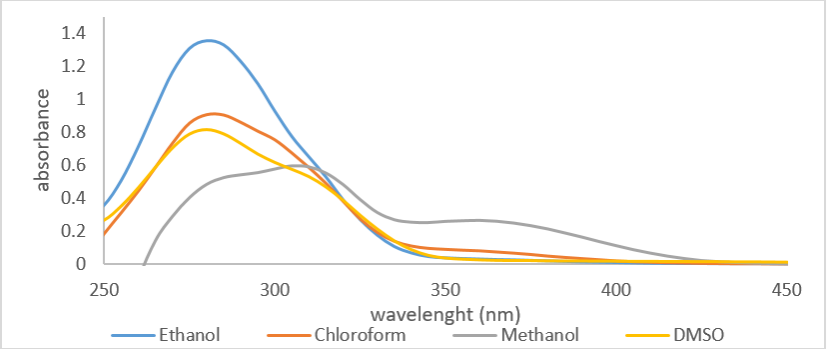


Fig. 3. Electron absorption spectra of Schiff base (A_3) in different organic solvents

Each of (A_1), (A_2) and (A_3) was gave different values of λ_{max} using different solvents, (Table 1) attributed to (π -π*) transition, which related to the aromatic system and (-HC=N-) group.

Table 1
The UV-visible spectrum of (A_1), (A_2) and (A_3)

No.	Base	Solvents	(CT) π→π* λ max (nm)	(CT) π→π* ε_{max} x10^4 l.mol^{-1}.cm^{-1}	(C=N) π→π* λ max (nm)	(C=N) π→π* ε_{max} x10^4 l.mol^{-1}.cm^{-1}	(Ar) π→π* λ max (nm)	(Ar) π→π* ε_{max} x10^4 l.mol^{-1}.cm^{-1}
1	A_1	Chloroform	----	----	308	0.56	270	0. 95
2		DMSO	----	----	310	0.88	275	1.25
3		Ethanol	----	----	310	0.79	270	0. 94
4		Methanol	----	----	308	0.78	275	1.26
1	A_2	Chloroform	----	----	300	0.9	275	1.3
2		DMSO	370	0. 46	310	0.7	280	0.68
3		Ethanol	----	-----	305	0.77	275	1.05
4		Methanol	----	-----	310	0.43	275	0.87
1	A_3	Chloroform	360	0.09	300	0.75	280	0.9
2		DMSO	----	----	315	0.46	280	0.81
3		Ethanol	----	----	---	----	280	1.35
4		Methanol	360	0.27	310	0.59	285	0.52

The table above displays the solvents effect in each base. The (A_1), (A_2 and A_3) were showed two and three absorption peeks in (200-500) nm respectively contributed to the different electronic transitions using different solvents. These results were indicated that the Schiff bases were affected by the solvation and the dielectric constant (D), which can have expressed by the relation of Gati and Szalay (Gaber, et al., 2019), as below:

$$\Delta\tilde{\nu} = [(a - b)\,(n^2 - 1 / 2n^2 + 1)] + b\,(D - 1 / D + 1)$$

The F(D) and Φ(D) were also calculated as seen in Table 2 below, the results were gave a linear relationship when the dielectric constant is the only effect controlling the beak shift.

$$F(D) = 2(D - 1) / (2D + 1) \quad \& \quad \Phi(D) = (D - 1) / (D + 2)$$

Table 2.
The values of the solvents, the dipole moment constants and the maximum wavelengths of (A_1), (A_2) and (A_3).

The Solvent	D	(D-1)/(D+1)	λ_{max} nm A_1	λ_{max} nm A_2	λ_{max} nm A_3
Chloroform	9.1	0.802	270 (s), 308 (m)	275 (s), 300 (w)	280 (m), 300 (w)
DMSO	47	0.958	275 (s), 310 (m)	280 (m), 305 (m), 370 (m)	280 (m), 315 (w)
Ethanol	24.3	0.921	270 (s), 310 (m)	275(s), 305 (m)	280 (s)
Methanol	32.7	0.94	275 (s), 308 (m)	275 (s), 310 (w)	285 (w), 310 (s), 360 (m)

W = Weak, m = Medium, s = Strong

The results, (Figures 13 - 14) also indicated, that there is no deviation from the linear relationship, which is due to the fact that the effect of the dielectric constant is the main factor that can control the shift of the absorption beaks with a slight deviation from the linearity, and this may effected by the hydrogen bond between the base and the solvent (Hammud, et al., 2008).

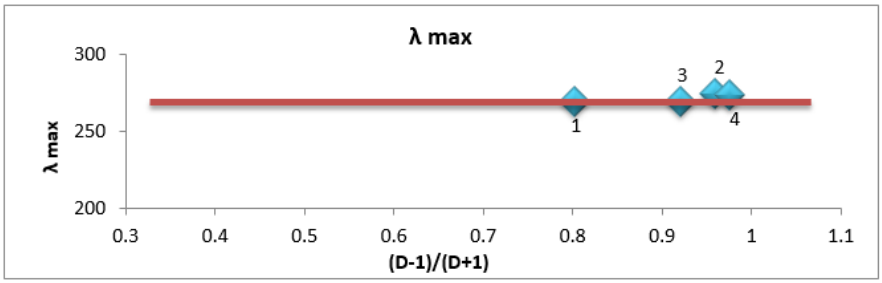


Fig. 4. The relation between the dipole moment constants and the maximum wavelengths of (A_1) using different solvents (1- Chloroform , 2- DMSO, 3- Ethanol, 4- Methanol.)

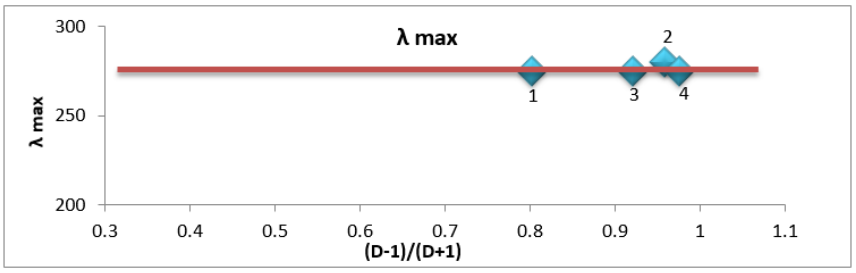


Fig. 5. The relation between the dipole moment constants and the maximum wavelengths of (A_2) using different solvents (1- Chloroform , 2- DMSO, 3- Ethanol, 4- Methanol.)

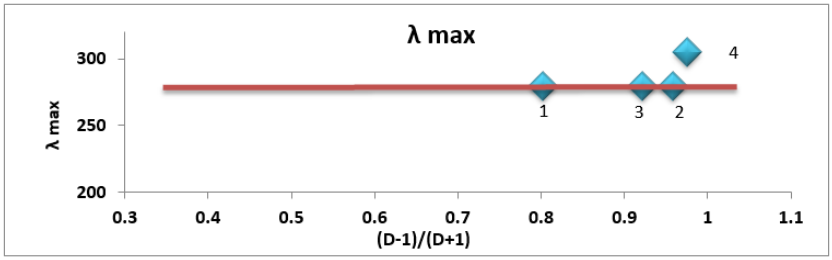


Fig. 6. The relation between the dipole moment constants and the maximum wavelengths of (A_3) using different solvents(1- Chloroform , 2- DMSO, 3- Ethanol, 4- Methanol.)

Further, the pH effect in the range of λ (250-500) nm was also studied for each schiff base (A_1), (A_2) and (A_3) in a range of buffer solution at pH (1-12) using $1*10^{-4}$ M as seen in Figures 19, 20 and 21 below:

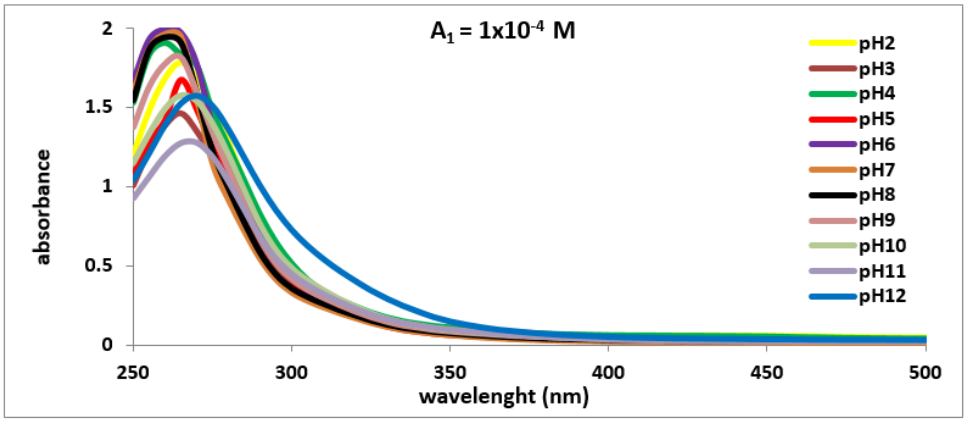


Fig. 7. Electron absorption spectra of Schiff base (A_1) in different pH solutions

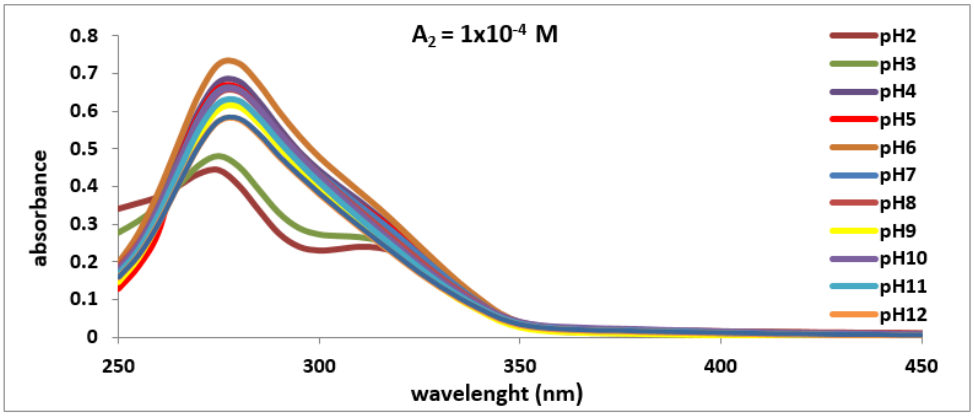


Fig. 8. Electron absorption spectra of Schiff base (A_2) in different pH solutions

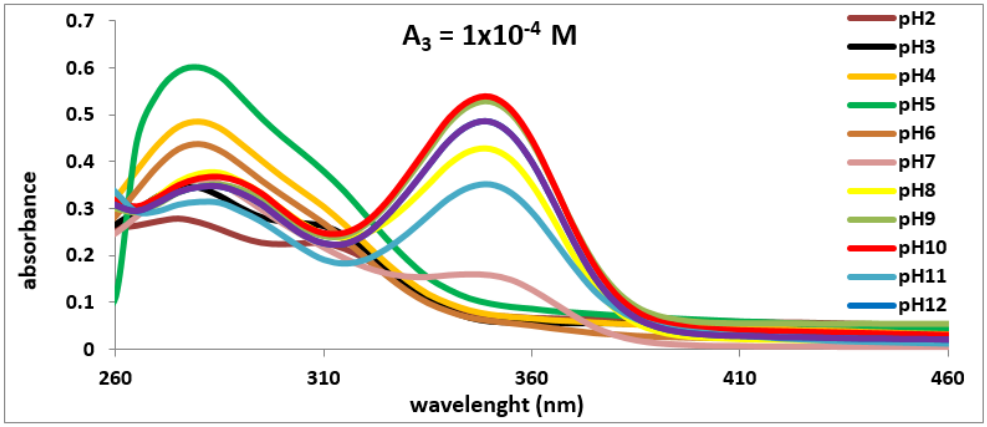


Fig. 9. Electron absorption spectra of Schiff base (A_3) in different pH solutions

The results were showed that the suitable pH values of (A_1), (A_2) and (A_3) were found to be in the pH6, pH2 and pH5 respectively. Two isopiestic points were gained in Figure 13. Therefore, the pKa of hydroxyl group and the pK_p of the nitrogen atom in the (A_3) were calculated by applying the half height method (Pretsch, et al., 2009). From this method, the pK values were attended using equations (1) and (2) below. This method was depending on the fact that the limiting absorption (Al) represents complete conversion of one form to other. Since pK is equal to pH at which the two forms exist in equivalent amount, then the pH corresponding to half the height of the absorbance, the pH curve is equal to pK.

pK = pH (at $A_{1/2}$) ..... (1)

$A_{1/2} = \frac{A_l + A_{min}}{2}$ ..... (2)

The pK at ($A_{1/2}$) of (A_3) was envisioned from the absorbance-pH curve as realized in Figure 14 below.

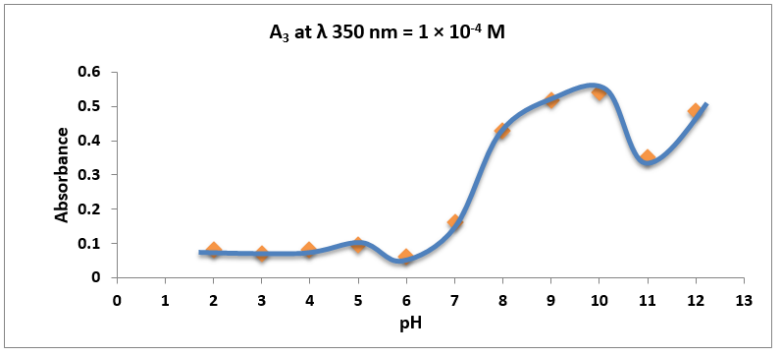


Fig. 10. Absorption curves - pH of base (A_3) at wavelength (350) nm

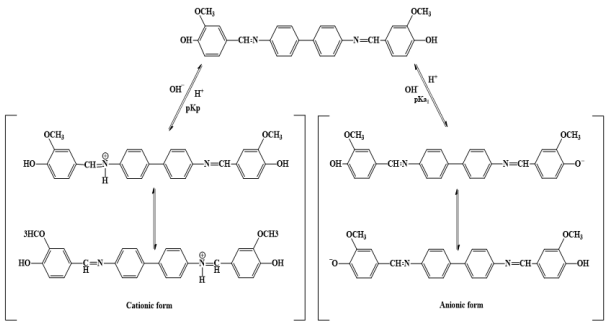
The results obtained from the absorbance-pH curve in figures above are given in Table 3 below.

Table 3
Spectrophotometric determination of ionization and protonation constants of (A_3).

Id		Base A_3 at λ = 350 nm		
		A_{min}	A_{max}	$A_{1/2}$
pKp	4.51	0.08	0.98	0.53
pKa	7.41	0.06	0.54	0.3

pKp = Protonation of the nitrogen atom
pKa = Ionization of the OH-group

The absorption spectra of (A_3) in the varying pH values are explained in the Schemes 2 and 3 below. The results were indicated the existence of the following equilibrium schemes of which displays the suggested ionization of (A_3) in acidic and basic medium.



Scheme 2 suggested Ionization and proton mechanics of the base (A_3).

The prepared Schiff bases (A_1, A_2, and A_3) were studied on breast cancer cells type (MCF-7) using five concentrations

(1000,500,250,125,62.5,31.25) µg/ml of each as shown in Figures 23-25 below.

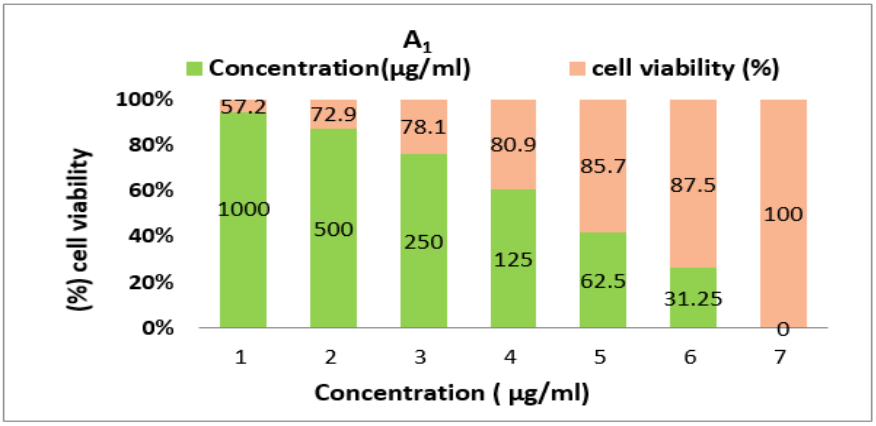


Fig. 11. The relationship of the percentage of inhibition with the concentration of (A_1)

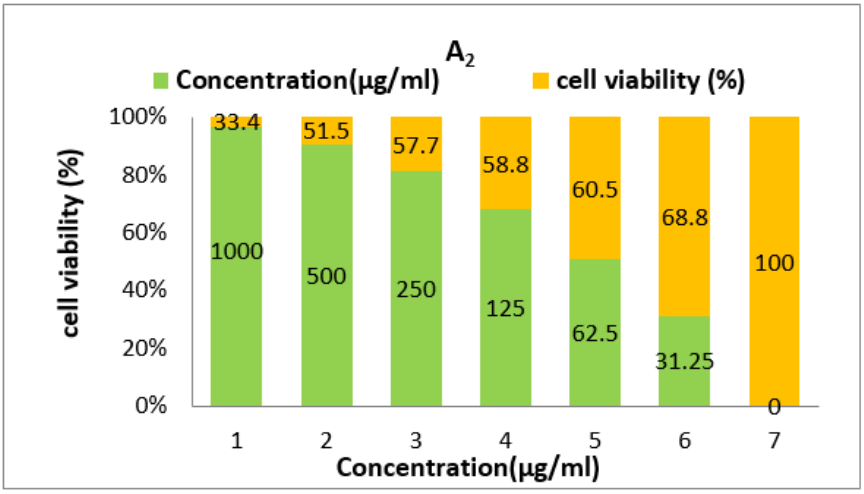


Fig. 12. The relationship of the percentage of inhibition with the concentration of (A_2)

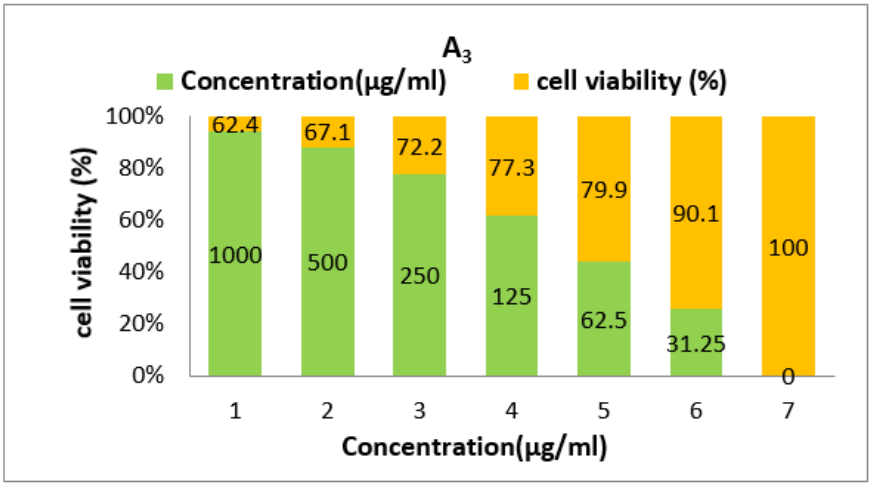


Fig. 13. The relationship of the percentage of inhibition with the concentration of (A_3).

The inhibition activity was showed that the three synthetic bases were possess an inhibitory ability at high concentration in variable values.

4. Conclusions

The three Schiff bases of mono-group (A_1) and di-azomethene (A_2 and A_3) were prepared. The prepared Schiff bases were identified using different spectrophotometric techniques and the results were identical to the proposed structures. The analytical study included the following: Studying the effect of the polarity of organic solvents and knowing the effect of dipole and solubility, and the values of the molar absorption coefficient and standard deviation of the prepared bases were calculated. Studying the effect of pH on the prepared bases, calculating the protonation and ionization constants, determining the isopiestic points of the base (A_3) and proposing the reaction mechanism for it. The bases showed high inhibition activity at high concentration due recommend these bases as novel anti-cancer drugs. Therefore, we recommend studying the pharmacological activity of the prepared compounds on other types of cancer cells, such as cancer Laryngeal and liver cancer. We also recommend the use of Schiff base compounds that contain hydroxyl substitutions in order to increase the effectiveness of anticancer drugs.

Competing Interests

The authors have declared that no competing interests exist.

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