Molecular Docking, Synthesis, Characterization and Biological Activity of New Azo-Schiff Bases Derivatives

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Abstract:

In this study, four Schiff base derivatives (S1-S4) were designed, synthesized and tested for biological activity. The synthesized compounds were analysed and described based on their physical and chemical properties, as well as through the use of ^{1}H-NMR and FTIR techniques. The molecular docking investigation demonstrated the potential biological action of these molecules.

The results showed that these compounds exhibit antibacterial, antifungal, and anticancer properties. Compounds S1 and S2 exhibited antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli*. Compounds S3 and S4 exhibited fungicidal properties against *Aspergillus niger* and *Chaladra corda*. Compound S3 demonstrated significant anticancer efficacy against breast cancer, as confirmed by MTT assay.

Key words: Schiff bases, molecular docking, anticancer, antibacterial, antifungal.

الارساء الجزيئي والتخليق والتشخيص والفعالية الحياتية لمشتقات جديدة قواعد شف ازو

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الخلاصة:

تم تصميم وتخليق وفحص الفعالية الحياتية لأربعة مشتقات قاعدة شف في هذه الدراسة. تم تحليل المركبات المخلقة وتثبيت صفاتها وخواصها الفيزيائية والكيميائية والتشخيص العضوي باستخدام تقنيات الرنين النووي المغناطيسي البروتوني NMR وطيف الاشعة تحت الحمراء الدقيق FTIR. أوضحت دراسة الارساء الجزيئي الفعالية الحياتية المحتملة لهذه الجزيئات. أوضحت النتائج ان هذه المركبات تمتلك واص مضادة للبكتريا والفطريات والسرطان. كما تبين امتلاك المركبين S1 وS2 تأثير مضاد لبكتريا *Staphylococcus aureus* و*Escherichia coli*. أما المركبين S3 وS4 فقد اثبت امتلاكها فعالية قاتلة لفطريات *Aspergillus niger* و *Chaladra corda*. وأخيرا اظهر مركب S3 تأثيرا مضادا لسرطان الثدي كما تم اثباته من خلال اختبار MTT.

الكلمات المفتاحية: قاعدة شف، الارساء الجزيئي، مضاد للسرطان، مضاد بكتيري، مضاد فطري.

Introduction:

Microbial infections represent a significant challenge to humankind. This is due to the evolving resistance against the potent antimicrobial agents. Therefore, efforts are continuous to design and develop new antimicrobial agents to combat antimicrobial resistance (1-3). In addition, cancer is the second leading cause of death after coronary heart diseases. It affects different organs of the human body mainly breast, prostate, intestine respiratory and gastrointestinal tract. There are many approaches to treat cancer. However, most of these drugs suffer from toxicity and are nonselective (4). Therefore, research is continuously growing to find novel safe and selective anticancer agents (4). Schiff bases have a crucial role as intermediates in producing bioactive chemicals, including β-lactams (5). In addition, these compounds have been documented to exhibit a range of intriguing biological effects, such as antibacterial (6,7), antifungal (7), inhibition of mouse hepatitis virus (MHV) (8), suppression of adenovirus type 5 (Ad 5) and herpes simplex virus type 1 (HSV-1) (9), anticancer (10, 11), and herbicidal activities (12). It is widely recognized that the inclusion of both an azo and a chloro functional groups in certain compounds can result in their ability to display pesticidal properties (13), Both Schiff bases and azo compounds play significant roles in the pharmaceutical industries. It has been proposed that the azomethine linkage in Schiff bases is responsible for their biological actions. Given the diverse range of biological activity observed in compounds possessing azo, methoxy groups, and azomethine linkages (14), it was deemed worthwhile to investigate the impact of having all of these functionalities present together in a single structure. Therefore, we select to synthesize 4 novel azo Schiff bases and evaluate their efficacy against Staphylococcus aureus and *Escherichia coli, Aspergillus niger* and *Chaladra corda*. Finally, it was decided to test these compounds against breast cancer cell lines.

Methods:

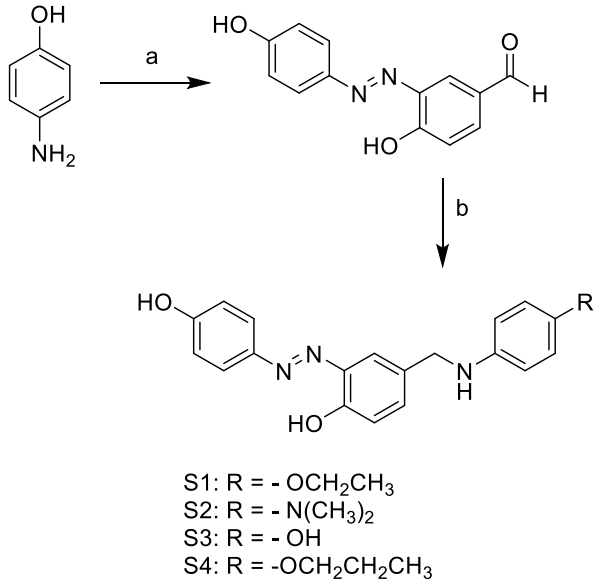
1- Materials

The materials used in this research are hydrochloric acid (HCl), 4-hydroxyaniline (C_7H_7NO), sodium nitrite ($NaNO_2$), 4-hydroxybenzaldehyde ($C_7H_6O_2$), sodium hydroxide (NaOH), ethanol (C_2H_6O), glacial acetic acid (CH_3COOH), 4-ethoxy aniline ($C_8H_{11}NO$), N^1, N^1-dimethylbenzene1,4-diamine ($C_8H_{12}N_2$), 4-hydroxyaniline (C_6H_7NO), and 4-propoxyaniline ($C_9H_{13}NO$).

2- Instruments

The instruments used in the research are the proton nuclear magnetic resonance spectrophotometer (^{1}H-NMR, Bruker, Germany) and FT-IR (Bruker, Germany).

3- Synthesis



Scheme 1. Synthesis of compounds S1-S4. (a) 4-Hydroxybenzaldehyde, $NaNO_2$, HCl, 0-5 °C, (yield 88%); (b) 4- substituted aniline, glacial acetic acid, ethanol, room temperature, (yields S1, 76%; S2, 69%; S3, 72%; S4, 78%)

(E)-4-hydroxy-3-((4-hydroxyphenyl) diazenyl) benzaldehyde (compound B)

A 10% HCl solution was prepared in a cooling bath (0-5 °C) and added to 4-hydroxyaniline (1.09 g, 10 mmol). Conc. HCl (2-3 drops) and $NaNO_2$ (10 mmol) in distilled water (5 ml) were added. NaOH solution (10%) was added to the solution of 4-hydroxybenzadehyde (1.22 g, 10 mmol). The solution was then filtered, concentrated and the precipitate was collected yielding a white powder (2.13 g, 88%). m.p. 176 ºC. IR (KBr) ν / cm^{-1} 3396, 3095, 2758, 1718, 1594, 1548, 1515, 1401, 1253, 1188, 1068, 890.

Synthesis of Schiff base derivatives (S1-S4)

Dissolve the azo derivative (B) (242 mg, 1 mmol) in 25 ml of 99.9% EtOH and add 3 drops of glacial acetic acid. The materials 4-ethoxy aniline, N1, N1-dimethylbenzene-1,4-diamine, 4- hydroxyaniline, and 4-propoxyaniline respectively and separately to form 4 solutions. The precipitates were filtered to and recrystallized from ethanol to yield the Schiff base derivatives.

4-((E)-((4-ethoxyphenyl) imino) methyl)-2-((E)-(4-hydroxyphenyl) diazenyl) phenol (S1)

Light yellow powder (274 mg, yield 76%). m.p. 231-233 °C. IR (KBr) ν / cm^{-1} 1639, 3050, 2963, 1600, 1509. ^{1}H NMR (400 MHz, d_6-DMSO) δ 1.43 (t, 3H, CH_3), 3.88 (q, 2H, CH_2), 6.91-8.08 (m, 11H, ArH), 8.83 (s, 1H, imine), 8.38 (s, 1H, OH), 9.22 (s, 1H, OH).

4-((E)-((4-(dimethylamino) phenyl) imino) methyl)-2-((E)-(4-hydroxyphenyl) diazenyl) phenol (S2)

Dark yellow powder (248 mg, yield 69%). m.p. 201-203 °C. IR (KBr) ν / cm^{-1} 1641, 3024, 2944, 1594, 1504. ^{1}H NMR (400 MHz, d_6-DMSO) δ 1.43 (s, 6H, $(CH_3)_2$), 6.86-7.67 (m, 11H, ArH), 8.81 (s, 1H, imine), 8.31 (s, 1H, OH), 9.39 (s, 1H, OH).

2-((E)-(4-hydroxyphenyl) diazenyl)-4-((E)-((4-hydroxyphenyl) imino) methyl) phenol (S3)

Dark yellow powder (240 mg, yield 72%). m.p. 188-190 °C. IR (KBr) ν / cm^{-1} 1637, 3072, 2926, 1600, 1520. ^{1}H NMR (400 MHz, d_6-DMSO) δ 6.86-7.67 (m, 11H, ArH), 8.60 (s, 1H, imine), 9.07 (s, 1H, OH), 9.38 (s, 1H, OH), 9.41 (s, 1H, OH).

2-((E)-(4-hydroxyphenyl) diazenyl)-4-((E)-((4-propoxyphenyl) imino) methyl) phenol (S4)

Dark yellow powder (292 mg, yield 78%). m.p. 235-237 °C. IR (KBr) ν / cm^{-1} 1644, 3039, 2960, 1591, 1514. ^{1}H NMR (400 MHz, d_6-DMSO) δ 1.10 (t, 3H, CH_3), 1.97-1.86 (m, 2H, CH_2), 2.96 (t, 2H, CH_2), 3.87 (s, 2H, OCH_2), (s, 2H, CH_2), 6.90-7.81 (m, 11H, ArH), 8.75 (s, 1H, imine), 8.29 (s, 1H, OH), 9.19 (s, 1H, OH).

Results and Discussion

1-Synthesis

The reaction starts with nitration of 4-hydroxybenzaldehyde using $NaNO_2$ in presence of HCl. This is followed by the formation of azo derivative (B). The final compounds (S1-S4) were obtained by the condensation of the azo derivative (B) with the corresponding aniline in presence of glacial acetic acid.

FT-IR spectroscopy: the FTIR spectroscopy gives the result of azo derivative (B) appearing as azo group at 1515 cm^{-1}. Diazonium salt is employed and undergoes as an electrophile and electrophilic substitution with an electron-rich coupling portion. In addition, the FTIR spectroscopy result of Schiff base derivatives (S1-S3) disappeared the carbonyl and C-H of aldehyde and appeared imine group of the new Schiff bases produced. for FTIR (cm^{-1}) results of S1-S4, respectively, it was showed that the imine group of Schiff base at (1639, 1641, 1637 and 1644) and disappeared the carbonyl group of the aldehyde group, while the C-H aromatic ring appeared at (3050, 3024, 3072 and 3039) and C-H of aliphatic group at (2963, 2944, 2926, and 2960). The C=C of the aromatic ring appeared in (1600, 1594, 1600, and 1591). The azo group appeared in (1509, 1504, 1520 and 1514). NMR spectroscopy

^{1}H-NMR (ppm) spectroscopy of derivative S1 showed a singlet signal of the proton of the two hydroxyl groups at 9.22 and 8.38 and a singlet signal of the proton of the imine group (1H) at 8.83. Multiple signals for protons of the aromatic ring (11H) at 6.91-8.08, while the protons of methyl group (3H) showed at 1.43 as triplet signals s and protons of CH_2 group quartet signal at 3.88. ^{1}H-NMR (ppm) spectroscopy of derivative S2 showed a singlet signal of the proton of the two hydroxyl groups at 9.39 and 8.31 and a singlet signal of the proton of the imine group at 8.81. Multiple signals for protons of the aromatic ring (11H) at 6.86-7.67, while the protons of two methyl groups (6H) showed at 2.97 as singlet signals.

^{1}H-NMR (ppm) spectroscopy of derivative S3 showed a singlet signal of the protons of the three hydroxyl groups at 9.07, 9.38 and 9.41 and a singlet signal of the proton of the imine group at 8.60. Multiple signals for protons of the aromatic ring (11H) at 7.09-7.86.

^{1}H-NMR (ppm) spectroscopy of derivative S4 showed two singlet signals of the protons of the two hydroxyl groups at 9.19 and 8.29 and a singlet signal of the proton of the imine group at 8.75. Multiple signals for protons of the aromatic ring (11H) at 6.90-7.81, while the protons of a methyl group (3H) showed at 1.10, the CH_2-$C\underline{H_2}$-CH_3 appeared as a multiplet at 1.79-1.86 and 2.96 as triplet signals and protons of O-CH_2 (2H) appears as singlet at 3.87.

2-Antibacterial activity

The antibacterial evaluation of the Schiff bases at a dosage of 50 mg/ml has yielded data indicating their effectiveness against all microorganisms. The diameter of the inhibitory zones was measured in

millimetres, and the corresponding findings are shown in Table 1. It was noted that only derivatives S1 and S2 has significant antibacterial activity. The antimicrobial testing results demonstrate that Schiff base derivatives exhibit notable activity against *Staphylococcus aureus*, and *Escherichia coli*. Derivative (S2) was particularly effective against all tested bacterial strains due to a nitrogen atom with two methyl group in its structure, which possesses inherent antimicrobial properties (15). It is suggested that the ethoxy substituent (S1) and dimethyl amino substituent (S2) have a good fitting with a hydrophobic pocket in the target while S3 has only hydroxyl group which is polar and hydrophilic group. On the other hand, although compound S4 has a hydrophobic substituent, it is thought that its size is mor bulky to fit on the hydrophobic pocket on the ligand. The antibacterial activity of these chemicals demonstrates an increasing order. As concentration increases, the region of growth inhibition likewise increases. These results were comparable to previous study conducted by Muhammad Ali and coworkers. The study reported the synthesis of 6 sulphonamide derivatives containing azo and Schiff bases portions. Most compounds demonstrated good antibacterial activity against *Staphylococcus aureus* with the best inhibition zone of 34 mm (15). The antibacterial activity against *Staphylococcus aureus* was compared against some known standard drugs extracted from a study carried out by Nikokar and his colleagues (16). On the other hand, findings related to *E. coli* were compared to sensitivity test with various antibacterial drugs conducted by Aditi and his colleagues as shown in table (1) (17).

Table (1): Antibacterial activity of derivatives (S1 and S2).

Derivative	Zone of inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
S1	24±1	19±1
S2	31±1	25±2
Penicillin G	32±1*	>28**
Clindamycin	21±1*	>21**
Gentamicin	19±1*	>15**
Erythromycin	22±1*	>23**
Ciprofloxacin	24±1*	>21**
Rifampicin	25±1*	>20**

* Findings from reference (16), ** Findings from reference (17).

3-Anti-fungal activities

The study results of the antifungal activity of the synthesized derivatives at a concentration of 50 mg/ml demonstrate that the derivative (S4) exhibits the highest level of effectiveness against all types of tested fungus. In addition, derivative S3 also has antifungal activity as shown in table 2. These findings were compared to amphotericin B which is a standard antifungal drug for both *Aspergillus* and *Chalara* species (18-19). The remaining derivatives do not demonstrate noteworthy antifungal activity. Previous research provides similar values of the antifungal inhibition zones caused by certain azo Schiff base compounds which ranged from 13-25 mm for *Aspergillus niger* (20).

Table (2): Antifungal activity of derivatives (S3 and S4).

Derivative	Zone of inhibition (mm)	
	Aspergillus niger	*Chalara corda*
S3	24	19
S4	31	25
Amphotericin B	> 16*	> 15**

* Findings from reference (18), ** Findings from reference (19).

4- Viability Assay by MTT method

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was used to determine the cytotoxicity of the Schiff base derivative (S3) against breast cancer cell lines. It is thought that the reason that compound S3 is the only derivative which has anticancer activity is the presence of hydroxyl group which is a hydrogen bond donor which is probably an essential group for hydrogen bonding interaction with the target. The cell viability was assessed after 24 and 48 hours of treatment with different dosages ranging from 0 to 320 ppm. The derivative (S3) findings are shown in Figure 1, indicating a range of 19–100 ppm at 24 hours and 13–100 at 48 hours. The results display a correlation between the dosage administered and the response seen in the breast cancer cell line. The selection of these compounds was based on their capacity to inhibit the COX-1 and COX-2 enzymes, making them very promising candidates for anti-cancer medications. The effect of the derivative (S3) on the viability of breast cancer cells was evaluated, as seen in Figure 1. Following a period of 48 hours, increased concentrations (ppm) of the derivative led to a further reduction in the viability of breast cancer cells, surpassing the loss reported after 24 hours. The pattern of these values was shown on other Schiff compounds and their effect on viability of certain breast cancer cell lines such as those synthesized by Hashim and Saad study (21).

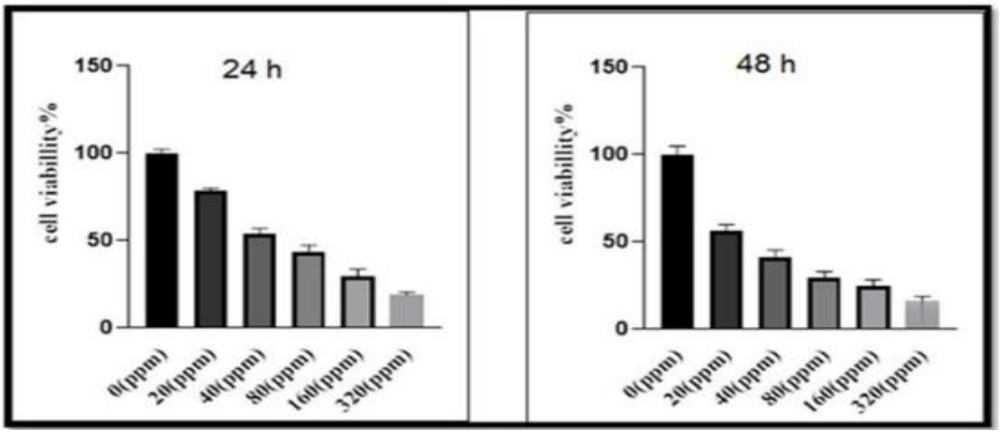


Figure (1): Effect of Schiff base derivative (S3) on breast cancer cell viability.

Table (3): The effect of Schiff base derivative (S3) on % viability of breast cancer cell line

Concentration (ppm)	After 24 h		After 48 h	
	Mean	SD	Mean	SD
0	100	2.807589	100	3.248782
20	75.8563	2.136022	55.6401	2.464002
40	51.2018	3.242472	42.2135	2.772486
80	43.4339	2.620165	23.1847	2.156913
160	28.7649	1.442438	19.3537	1.197497
320	19.1394	1.353564	13.8263	1.807724

5- Molecular Docking

The docking simulations have a major role in exploring the binding modes of ligands with a target molecule. Glucosamine-6-phosphate synthase (GP6 synthase), has attracted the interest of several researchers due to its importance in microbial cell wall synthesis. The enzyme catalyses the first step in hexosamine biosynthesis and converted fructose-6-Phosphate into GlcN-6-P (glucosamine6-phosphate), which is considered a precursor of uridine diphosphate N-acetyl glucosamine (UDPNAG), an essential component of the peptidoglycan layer of the microbial cell wall (22). The docking profiles of ligands are demonstrated below, the derivatives S1, and S2 are showing good binding performance (23), with both ligands formed multiple interactions with GP6 receptor S1 have slightly higher binding score -7.3 kcal/mol compared to S2 derivative which has -7.1 kcal/mol the difference can be attributed to S1 having more binding interactions with active site amino acids of the enzyme as shown in Figures 2-3.

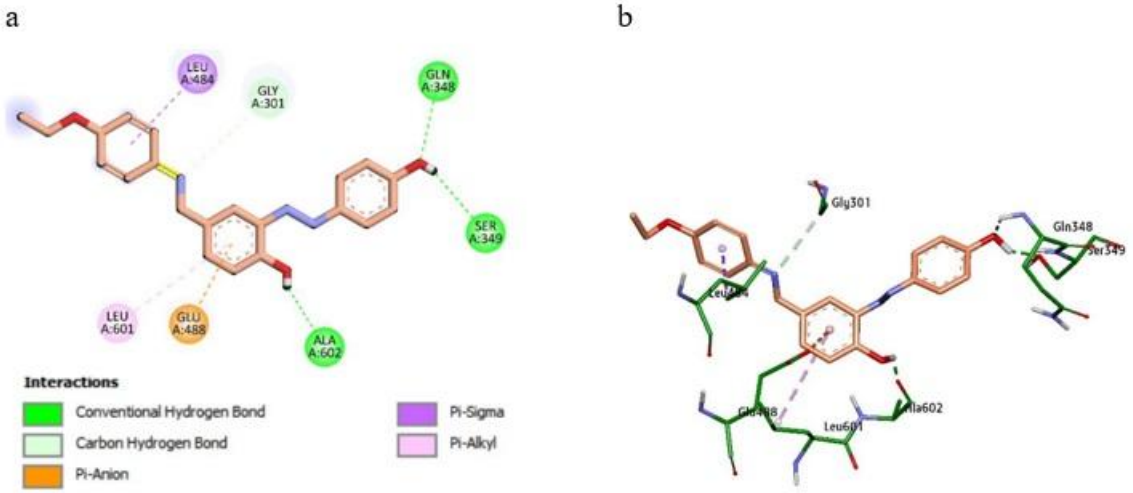


Figure (2). Molecular docking of compound S1 (a) 2D, (b) 3D

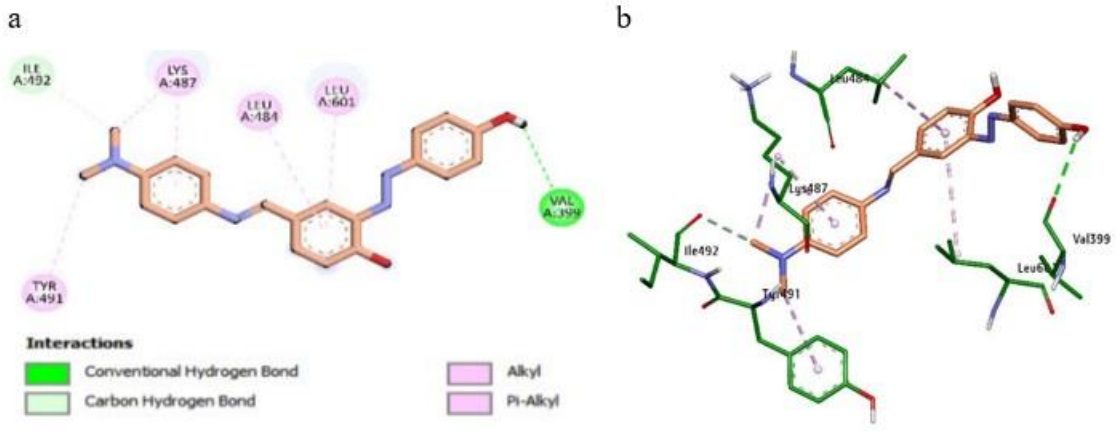


Figure (3). Molecular docking of compound S2 (a) 2D, (b) 3D

Human androgen receptor (AR) is one of main therapeutic target for prostate cancer (PCa) (23). The synthesized derivative exhibits a noticeable potency inhibition at AR's binding site (PDB:1E3G) with binding score (-8.5) kcal/mol. Figure 4 showed the interactions between the ligand and amino acids that located at receptor's binding pocket. The synthesized derivative demonstrates multiple interactions including (hydrogen bonding between GLN711 and hydroxy group of the compound, pi-alkyl was observed between aromatic ring of compound with PRO682 & ALA748).

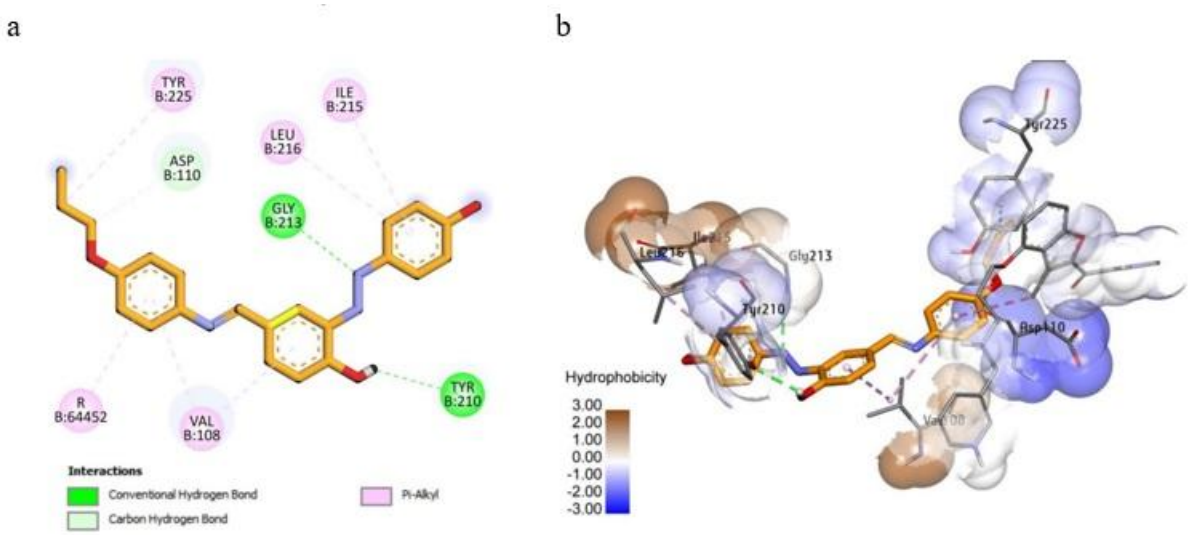


Figure (4). Molecular docking of compound S3 (a) 2D, (b) 3D

Myristoyl-CoA: protein N-myristoyltransferase (NMT) is a cytosolic monomeric enzyme that catalyzes the transfer of the myristoyl group from myristoyl-CoA to the N-terminal glycine of a number of eukaryotic cellular and viral proteins. NMT is a promising target enzyme for the development of novel fungicidal drugs having a broad antifungal spectrum. In the docking figures, the ligand is showing multiple interactions with the protein receptor most worth mentioning are two hydrogen bonding formed between GLY213 and azo group while second one formed between TYR210 and hydroxy group of the ligand with docking score -7.4 kcal/mol, as shown in figure 5 (24).

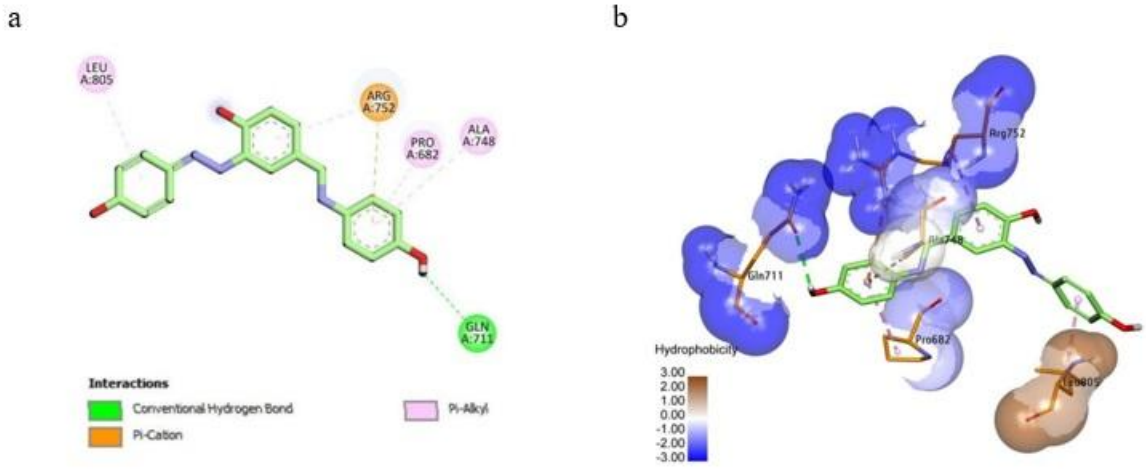


Figure (5). Molecular docking of compound S4 (a) 2D, (b) 3D

Conclusions

In conclusion, four azo-Schiff base derivatives (S1-S4) were successfully synthesized. Derivatives S1 and S2 showed a good zone of inhibition against *S. aureus and E. coli*. In addition, compounds S3 and S4 demonstrated antifungal properties. and evaluated as antibacterial, antifungal and anticancer agents. Furthermore, compound S3 has a good anticancer activity against breast cancer cell lines. Finally, molecular modelling analysis revealed the proposed mode of binding with their targets. It can be concluded that these compounds will have a promising pharmacological activity.

Conflicts of interests

The authors declare no conflict of interests.

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