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Original Article

Synthesis, Antibacterial Evaluation, and Docking Studies of Some Azo Compounds and Schiff Bases Derived from Sulfonamide

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ABSTRACT

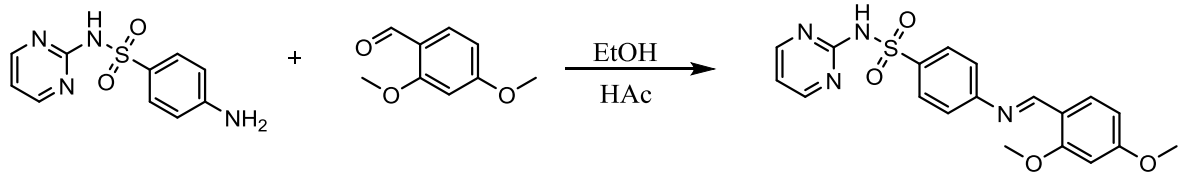
Objective: The aim of study was to synthesize new products of sulfonamide compounds containing azo and Schiff base fragments and confirm the structures by ^{1}H-NMR and FT-IR spectroscopy, as well as to investigate the antibacterial activities against medically important Gram (+) and Gram (-) bacterial strains.

Materials and Methods: Novel sulfonamides derivatives S1, S2, S3, A1, A2, and A3 were synthesized and tested with *staphylococcus aureus* and *Pseudomonas aeruginosa* as well as against *Candida albicans* fungi. Molecular docking was used to study the theoretical binding of the compounds with some selected proteins.

Results: It was found that compounds S1, S2, and S3 have more potent activity against the three types of microorganisms as compared with A1, A2, and A3. Against *Candida albicans*, it was found that compounds S1 and S3 gave the best activity, 21 and 20 mm, respectively. Antibacterial activity showed that compound A2 gave the best activity (34 mm at 1000 µg/mL) against *Staphylococcus aureus*. Other compounds S1, S2, S3, A1, and A3 gave very good activities against the same bacteria, 29, 13, 29, 28, and 28 mm, respectively, at the same concentration. Antibacterial activity against *Pseudomonas aeruginosa* showed that the compounds S1 gave the best inhibition zone (25 mm) at 1000 µg/mL, whereas compounds S2 and S3 showed good potent activity (15 and 20 mm, respectively). Molecular docking studies showed that free binding energy (S) of the compounds against *S. aureus* using protein 1JIJ were -7.15 to -8.60 kcal/mol, whereas free binding energy (S) using 5V5Z fungi protein gave the values -7.10 to -8.22 kcal/mol.

Conclusion: Schiff bases gave good activity against the selected microorganism species compared with azo compounds. There is clear correlation between the activity of the compounds and their molecular docking through the high negative values of free binding energy.

GRAPHICAL ABSTRACT



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Introduction

Sulfonamides are a significant group of synthetic bacteriostatic antibiotics that are still employed today to treat bacterial infections as well as infections brought on by other microorganisms [1]. Sulfonamides have mostly been supplanted by alternative treatments, but they still have a significant impact on the treatment of some infections, such as those of the bronchitis, eye, and ear infections, and urinary tract infections [2]. Schiff bases, which may synthesized from sulfonamide and aldehydes, having a broad range of biological effects in clinical medicine, are also referred to as anticancer and antiviral medicines. The antibacterial action is caused by the presence of azomethine and the sulfonamide functional group, and it can be changed based on the kind of substituents that are present on the aromatic rings [3]. On the other hand, azo dyes sulfonamide may synthesize from coupling between sulfonamide and electron donating aromatic systems. Therefore, many drugs containing azo-sulfa as antibacterial medications were among the first powerful chemotherapeutic treatments that could be used systemically to treat bacterial infections in people [4]. A series of azo dyes containing the sulfonamide functional group were synthesized as potential antimicrobial agents [5].

The essential bacterial mechanism for the manufacture of dihydrofolic acid is disrupted by the broad-spectrum synthetic pharmacological compounds, which compete with and inhibit the binding of p-amino benzoic acid (PABA) in the binding site of dihydropteroate synthase (DHPS) [6]. Folic acid is a crucial component needed for the DNA and RNA synthesis in bacteria. Sulfonamides impair bacteria's capacity to divide and reproduce by interfering with the synthesis of folic acid in bacterial cells. Sulfonamides and their derivatives are frequently prescribed for their antibacterial, antiviral, antifungal, anti-cancer, and anti-inflammatory properties [7, 8].

Here, we present information in this article on six new sulfonamide derivatives, three of which containing imine moiety and other three containing azo moiety. The study includes information on their mode of synthesis, physical and chemical characteristics, structural information, and binding affinities, with antibacterial and antifungal activity.

Martials and Methods

Materials and Reagents

All of the compounds employed in this investigation were of the reagent grade and were not further purified. They were provided by either Sigma-Aldrich or Fluka.

Characterization

Capillary tubes with a single side sealed were used to measure uncorrected melting points. At Tehran University in the Islamic Republic of Iran, ^{1}H-NMR spectra were scanned using a 500 MHz Bruker model Ultra Shield (Switzerland). Shimadzu model 8400 FT-IR at the Department of Chemistry, College of Science, and a spectrophotometer was utilized to record the FT-IR spectra using KBr discs.

Synthesis of Schiff base series

Compounds of Schiff bases series were synthesized by condensation of a solution of sulfonamide compounds 0.01 mole (2.5 g; sulfadiazine, 2.78 g; sulfamerazine, and 2.14 g; sulfaguanidine) in 50 ml warm ethanol and few drops glacial acetic acid with solution of 2,4-dimethoxybenzaldehyde 0.01 mole (1.66 g) in ethanol. The reaction mixture was refluxed for 4 hours, and then few formed precipitates were stirred at room temperature for further 2 hours to complete reaction. The product was filtered and recrystallized from ethanol, and then dried at room temperature [9], as displayed in Scheme 1.

*(E)-4-((2,4-dimethoxybenzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide (**S1**)*

Pale yellow, 85% yield, mp 190-193 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3411 (N-H), 3010 (C-H, aromatic), 1619 (C=N), 1532 (C=C), 1234, 1132 (C-N, C-O). ^{1}H-NMR (DMSO-d_6): δ 11.53 (s, 1H, NH), 8.36 (s, 1H, -CH=N-), 6.34-8.87 (m, 10H, Ar-H), 3.87 (s, 6H, -O-CH_3). Analysis: calcd (found) for $C_{19}H_{18}N_4O_4S$ (298.44): C, 57.28 (56.89), H, 4.55 (4.37), N, 14.06 (13.92), S, 8.05 (8.12).

*(E)-4-((2,4-dimethoxybenzylidene)amino)-N-(4,6-dimethylpyrimidin-2-yl)benzene sulfonamide (**S2**)*

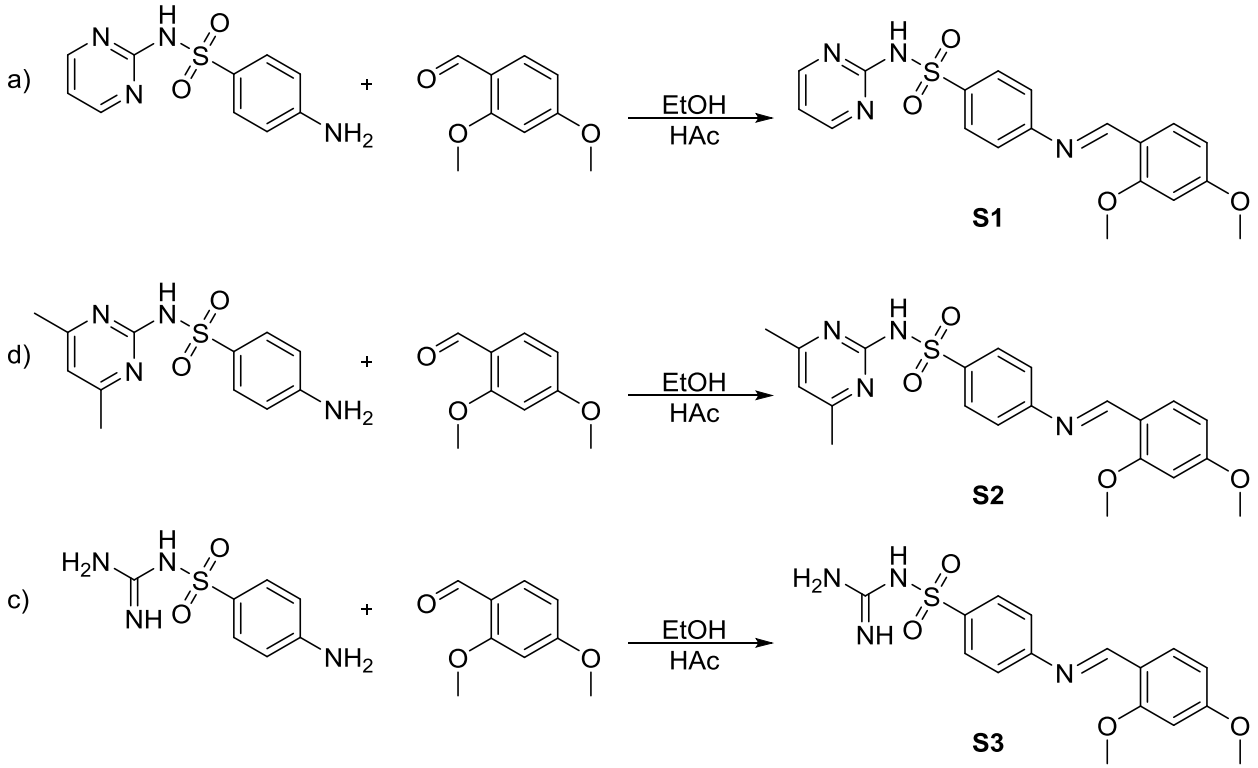
Yellow, 83% yield, mp 180-182 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3373 (N-H), 3010 (C-H, aromatic), 1595 (C=N), 1505, 1424 (C=C), 1208, 1152 (C-N, C-O). ^{1}H-NMR (DMSO-d_6): δ 11.23 (s, 1H, NH), 8.28 (s, 1H, -CH=N-), 6.65-8.27 (m, 10H, Ar-H), 3.74 (s, 6H, -O-CH_3), 2.85 (s, 3H, -CH_3). Analysis: calcd (found) for $C_{21}H_{22}N_4O_4S$ (426.49): C, 59.41 (59.29), H, 5.20 (5.29), N, 13.14 (13.12), S, 7.52 (7.42).

*(E)-N-carbamimidoyl-4-((2,4-dimethoxy benzylidene)amino)benzenesulfonamide (**S3**)*

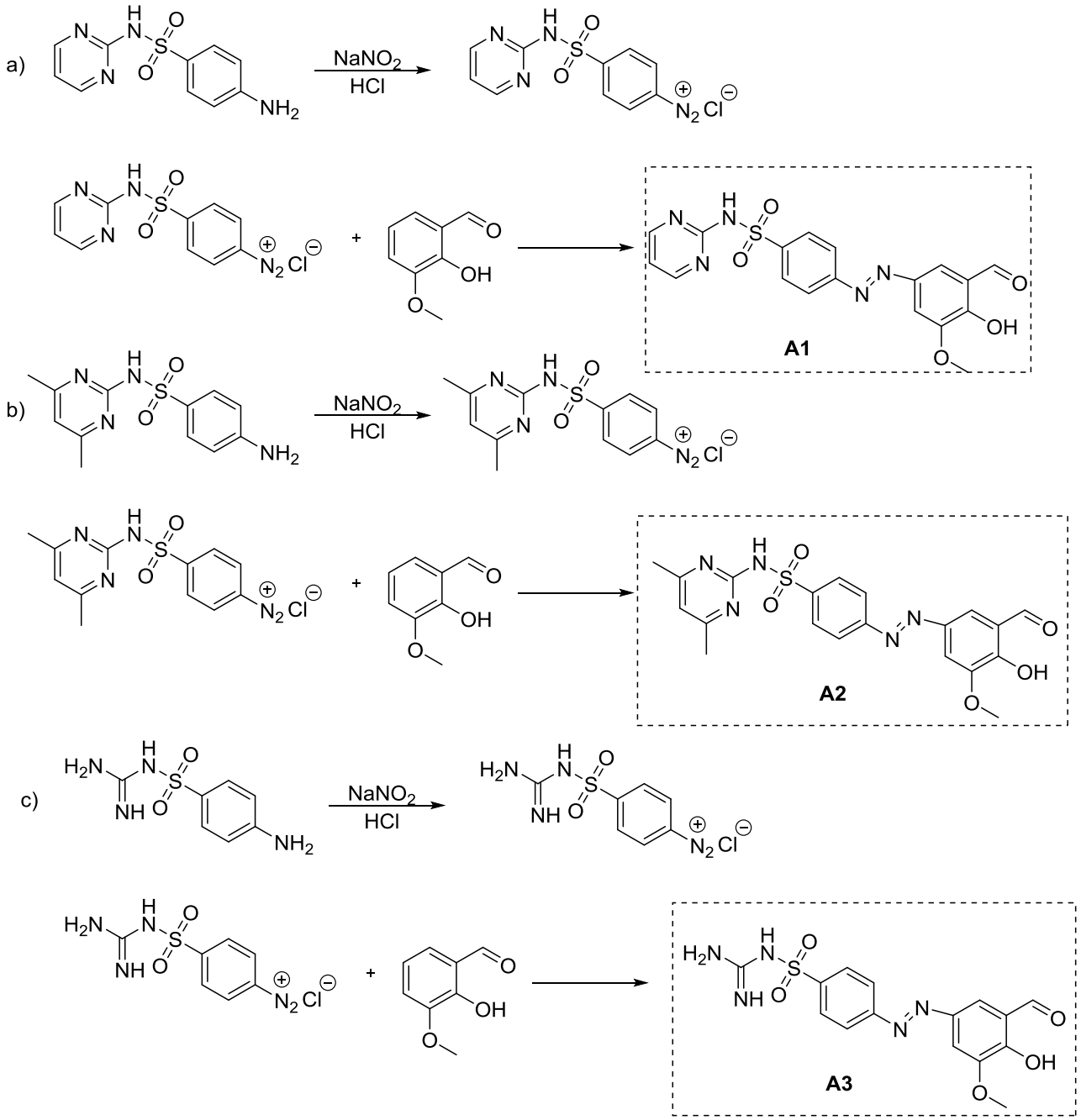
Pale yellow, 80% yield, mp 213-215 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3355 (N-H), 3039 (C-H, aromatic), 1651 (C=N), 1581, 1493 (C=C), 1262, 1156 (C-N, C-O). ^{1}H-NMR (DMSO-d_6): δ 11.46 (s, 1H, NH), 8.31 (s, 1H, -CH=N-), 6.57-8.56 (m, 10H, Ar-H), 6.48 (s, 4H, N-NH_2), 3.81 (s, 6H, -O-CH_3). Analysis: calcd (found) for $C_{16}H_{18}N_4O_4S$ (362.40): C, 53.03 (53.31), H, 5.01 (4.99), N, 15.46 (15.38), S, 8.85 (8.94).

Synthesis of azo series

Sulfonamides 10 mmol (2.5 g; sulfadiazine, 2.78 g; sulfamerazine, and 2.14 g; sulfaguanidine), water (10 mL), and concentrated hydrochloric acid (30 mmol) were combined, and the mixture was agitated until a clear solution was produced. A solution of sodium nitrite, 11 mmol in 5 mL of water, was then added dropwise, keeping the mixture's temperature less than 5 °C, after cooling it to between 0 and 5 °C. The mixture was then agitated for a further hour. In 8 mL of an aqueous NaOH solution that had been cooled to 0-5 °C in an ice bath, vanillin 10 mmol (1.52 g) was dissolved. The diazonium chloride salt solution was then progressively added to this solution, and the resulting mixture was continuously agitated at 0-5 °C for 2 hours. Overnight was spent on the concoction. After being precipitated by acidification, the resulting crude product was filtered, washed numerous times in cold water, and then recrystallized from ethanol [10], as depicted in Scheme 2.



Scheme 1: Synthesis pathway of Schiff bases a) **S1**, b) **S2** and c) **S3**



Scheme 2: Synthesis pathway of azo compounds

*(E)-4-((2,4-dimethoxybenzylidene)amino)-N-(pyrimidin-2-yl)benzenesulfonamide (**A1**)*

Orange, 74% yield, m p >250 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3302 (O-H), 3091 (C-H, aromatic), 1664 (C=N), 1589, 1492 (C=C), 1454 (N=N), 1284, 1163 (C-N, C-O). ^{1}H-NMR (DMSO-d_6): δ 12.23 (s, 1H, OH), 11.46 (s, 1H, NH), 9.63 (s, 1H, -CHO), 6.54-8.47 (m, 9H, Ar-H), 4.12 (s, 3H, -O-CH_3). Analysis: calcd (found) for $C_{18}H_{15}N_5O_5S$ (413.41): C, 52.30 (52.53), H, 3.66 (3.61), N, 16.94 (17.03), S, 7.76 (7.62).

*(E)-4-((2,4- dimethoxybenzylidene)amino)-N-(4,6-dimethylpyrimidin-2-yl)benzene sulfonamide (**A2**)*

Red, 77% yield, mp >200 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3388 (O-H), 3157 (C-H, aromatic), 1618 (C=N), 1587, 1490 (C=C), 1460 (N=N), 1263, 1161 (C-N, C-O). ^{1}H-NMR (DMSO-d_6): δ 11.98 (s, 1H, OH), 11.38 (s, 1H, NH), 9.77 (s, 1H, -CHO), 6.61-8.37 (m, 9H, Ar-H), 4.03 (s, 3H, -O-CH_3), 2.88 (s, 3H, -CH_3). Analysis: calcd (found) for $C_{20}H_{19}N_5O_5S$ (441.46): C, 54.41 (54.23), H, 4.34 (4.28), N, 15.86 (15.78), S, 7.26 (7.38).

*(E)-N-carbamimidoyl-4-((2,4-dimethoxy benzylidene)amino) benzene sulfonamide (**A3**)*

Red, 78% yield, mp >250 °C, IR (KBr) (ν_{max}/ cm^{-1}): 3367 (O-H), 3057 (C-H, aromatic), 1616 (C=N), 1593, 1568 (C=C), 1463 (N=N), 1255, 1157 (C-N, C-O). ^{1}H-NMR (DMSO-d_6): δ 12.61 (s, 1H, OH), 11.24 (s, 1H, NH), 9.65 (s, 1H, -CHO), 6.73-8.29 (m, 9H, Ar-H), 6.56 (s, 4H, N-NH_2), 4.18 (s, 3H, -O-CH_3). Analysis: calcd (found) for $C_{15}H_{15}N_5O_5S$ (377.38): C, 47.74 (47.63), H, 4.01 (3.97), N, 18.65 (18.58), S, 8.50 (8.46).

Antimicrobial activity

Studies on antimicrobial agents were carried out using the agar diffusion method [11, 12]. All produced compounds were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* to learn more about the antibacterial potency of sulfonamide derivatives, and the least bactericidal concentration was established. All chemicals were dissolved in dimethyl sulfoxide under the test conditions listed below (DMSO). Plates of sensitize agar (nutrient agar for bacteria) and sensitize agar (SDA for fungus) were made and dried at 25 °C for around 30 min. Using sterile swap, test strains were distributed over a solid sensitize agar surface. A blank test revealed that the test organisms were unaffected by the DMSO used to prepare the test solutions. At 37 °C, they were allowed to incubate. The edge-to-edge confluent growth zone, which typically correlates to the zone's sharpest edge, was used to compute the inhibition zone surrounding the hole. Its diameter was measured at 6 millimeters, and 80 µL was added. Each test was conducted three times before the average data was used to determine the results.

Molecular docking studies

Molecular docking of the synthesized series of compounds was performed using MOE 2015 v10 and all water and ligand molecules were removed. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase (protein ID: 1JIJ) and *Candida albicans* (proteins ID: 5V5Z) was obtained from RCSB protein data bank.

Results and Discussion

FT-IR spectra

All chemicals exhibit medium to strong intensity absorption bands at 1595–1664 cm^{-1}, which are attributed to the C=N stretching mode. Aromatic rings (C=C) have been detected thanks to their distinctive ring vibrations in the 1593-1424 cm^{-1} area [13]. The proposed azo dyes framework is supported by the presence of medium-strong bands specifically for (N=N) for azo compounds in the region of 1454-1463 cm^{-1} for compounds A1, A2, and A3. The existence of the O-H in the vanillin fragment is shown by the broad to weak bands in the region of about 3300 cm^{-1} in the spectra of azo compounds. All compounds' spectra display bending vibration bands in the region around 1300 cm^{-1} that are attributable to the methyl groups in the -OCH_3 fragment [14].

^{1}H NMR spectra

To identify the structure of Schiff bases, the following signals at the range as singlet δ 11.53-11.23, as singlet δ 8.36–8.28, and as multiplet δ 8.87-6.34 ppm are due to NH of sulfonyl amide, azomethine proton, and Ar-protons, respectively [15], whereas at high field range, there is singlet signals attributed to aliphatic protons of methoxy groups which appeared at δ 3.87-3.74. On the other hand, methazine fragment of S2 gave aliphatic singlet signal at δ 2.85 ppm of methyl groups. The sulfaguanidine fragment of S3 gave singlet signal referred to six protons of terminal two NH_2 protons [16].

The azo compounds gave the common signals for sulfonamide fragment, singlet signal for amide proton, multiplet signal for aromatic proton, methyl protons as singlet for methazine part, and singlet signal for symmetrical protons of NH_2 of sulfaguanidine part. Whereas aldehyde fragment showed download field singlet signal at the range δ 9.77-9.53 ppm and singlet signal for hydrogen bonded OH at δ 12.23-11.98 ppm [17]. Finally, high field singlet signal at δ 4.18-4.03 ppm attributed to methoxy protons.

Antimicrobial activity

The activity of the compounds against the microorganisms was showed in Tables 1, 2, and 3. The antibacterial activity showed that the compounds gave very good activity against *S. aureus*, where the A2 showed the best activity (34 mm) than other compounds at 1000 µg/mL, as presented in Tab. 6. On the other hand, the Schiff bases compounds gave high inhibition zone (15-25 mm) against *Pseudomonas aerugionosa* at the highest concentration and against *Candida albicans* (13-21 mm), whereas azo compounds gave no inhibition against the same bacteria and against the fungi. In general, the activity of Schiff base was more potent than azo compounds and the inhibition zone of all potent compounds was proportional with the concentration.

Table 1: Inhibition zone (mm) of compounds A1-S3 on *Staphylococcus aureus*

Conc. (µg/mL)	Compound					
	A1	**A2**	**A3**	**S1**	**S2**	**S3**
1000	28	34	28	29	13	29
500	26	22	27	27	Nil	27
250	23	15	13	24	Nil	24
125	22	Nil	10	17	Nil	20

Table 2: Inhibition zone (mm) of compounds A1-S3 on Pseudomonas aerugionosa

Conc. (µg/mL)	Compound					
	A1	**A2**	**A3**	**S1**	**S2**	**S3**
1000	Nil	Nil	Nil	25	15	20
500	Nil	Nil	Nil	18	13	13
250	Nil	Nil	Nil	16	11	13
125	Nil	Nil	Nil	9	Nil	11

Table 3: Inhibition zone (mm) of compounds A1-S3 on Candida albicans

Conc. (µg/mL)	Compound					
	A1	**A2**	**A3**	**S1**	**S2**	**S3**
1000	Nil	Nil	Nil	21	13	20
500	Nil	Nil	Nil	16	12	15
250	Nil	Nil	Nil	14	9	Nil
125	Nil	Nil	Nil	Nil	Nil	Nil

Molecular docking studies

The best method for examining the interaction between a target protein and an inhibiting chemical is molecular docking analysis [18]. Using the program MOE (2015.10), docking studies were carried out. From the Protein Data Bank's website (www.rcsb.org), the protein's 3D crystallographic structure was retrieved (PDB ID: 1JIJ and ID: 5V5Z). On ChemDraw Ultra 16.0, all compounds' 2D structures were depicted.

The covalent docking of all synthesized compounds into the largest pocket of TyrRS enzyme in *S. aureus* and CYP51 enzyme in *C. albicans* was simulated. The predicted docking scores are listed in Table 4. Azo derivatives showed favorable docking scores ranging from -6.95 to -7.75 kcal/mol with TyrRS enzyme, whereas Schiff bases gave affinity in the range -7.15 to -8.60 kcal/mol with the same *S. aureus* protein (compared with amoxicillin -7.56 kcal/mol). On the other hand, the affinity of the synthesized compounds with CYP51 enzyme showed the scores in the range -6.61 to -8.44 kcal/mol (compared with fluconazole -6.11 kcal/mol). RMSD values for all synthesized compounds were between 1.33 and 2.34. Tables 5 and 6 display the findings for just the substances that produced reliable molecular docking scores.

Table 4: Docking scores of synthesized compounds covalently bound to the active site of TyrRS (1JIJ protein), CYP51 enzyme (5V5Z protein), and standard drugs

Compound	Docking affinity (kcal/mol)			
A1	1JIJ	2.19	5V5Z	RMSD Å
A2	-7.75	1.72	-7.31	1.67
A3	-7.72	2.15	-7.51	1.33
S1	-6.95	1.45	-6.61	1.93
S2	-8.20	1.38	-7.98	2.08
S3	-8.60	1.48	-8.44	2.34
Amoxicillin	-7.15	1.47	-7.02	2.14
Fluconazole	-7.56	-	-	-
	-		-6.11	1.90

Table 5. Molecular docking score, RMSD, and binding affinity for the compounds showed valid molecular docking scores with 1JIJ

Compound	S Score kcal/mol	RMSD Å	Bonds between Atoms of Compounds and Residues of Active Site of 1JIJ					
			Compound Atoms	Receptor Atoms	Receptor Residues	Interaction	d (Å)	E (kcal/mol)
A1	-7.75	2.19	N 3	OD2	ASP 195	H-donor	3.00	-6.8
A2	-7.72	1.72	N 3	OD1	ASP 195	H-donor	3.34	-0.6
			N 3	OD2	ASP 195	H-donor	3.26	-2.6
A3	-6.95	2.15	N 21	OG1	THR 42	H-donor	3.20	-1.1
			O 16	NZ	LYS 84	H-acceptor	3.06	-1.0
S1	-8.20	1.45	N 10	SG	CYS 37	H-donor	3.36	-0.8
			C 13	OD2	ASP 195	H-donor	3.44	-1.0
S2	-8.60	1.38	C 13	OD2	ASP 195	H-donor	3.52	-0.8
S3	-7.15	1.48	N 9	SG	CYS 37	H-donor	3.32	-1.4
			C 12	OD2	ASP 195	H-donor	3.31	-1.6
			N 17	O	PRO 222	H-donor	3.15	-3.2
			N 20	OD2	ASP 195	H-donor	3.11	-0.9

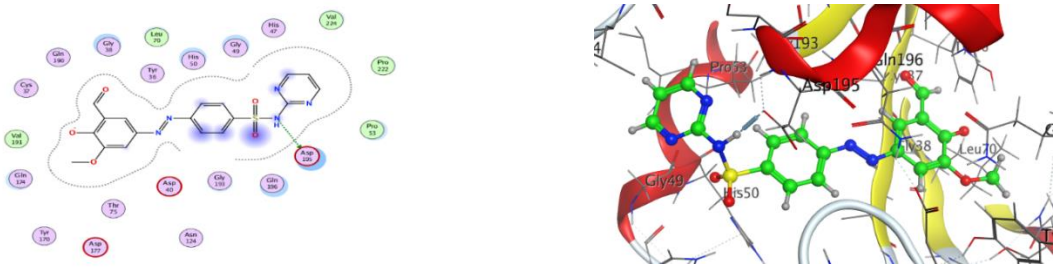
Table 6: Molecular docking score, RMSD, and binding affinity for the compounds showed valid molecular docking scores with 5V5Z

Compound	S Score kcal/mol	RMSD Å	Bonds between atoms of compounds and residues of active site of 5V5Z					
			Compound Atoms	Receptor Atoms	Receptor Residues	Interaction	d (Å)	E (kcal/mol)
A1	-7.31	1.67	N 3	O	TYR 87	H-donor	2.79	-2.6
			O 17	OH	TYR 47	H-acceptor	2.73	-1.8
A2	-7.51	1.33	O 16	SD	MET 50	H-donor	4.13	-0.3
			N 36	OH	TYR 47	H-acceptor	3.37	-1.2
A3	-6.61	1.93	N 3	O	TYR 87	H-donor	2.79	-2.6
			O 17	OH	TYR 47	H-acceptor	2.73	-1.8
S1	-7.98	2.08	O 29	OH	TYR 47	H-acceptor	2.74	-1.2
S2	-8.44	2.34	6-ring	CD1	LEU 15	pi-H	3.84	-0.7
			6-ring	CB	MET 35	pi-H	4.14	-0.6
S3	-7.02	2.14	6-ring	CD1	LEU 15	pi-H	4.10	-0.6

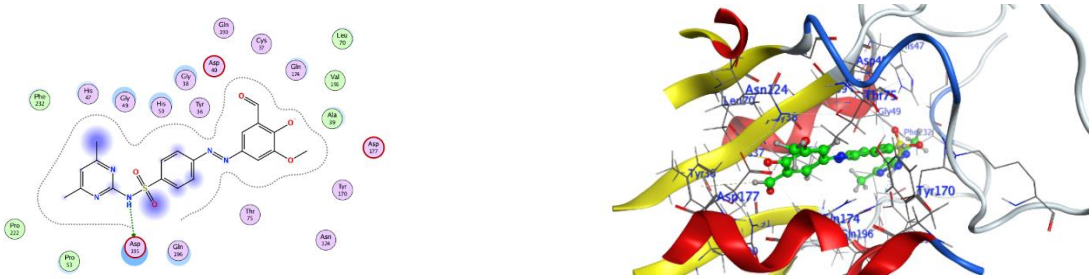
The best simulation of the synthesized compound with TyrRS enzyme and with CYP51 enzyme was S2 which showed greater value than standard drugs, as depicted in Figures 1 and 2.

The molecular docking of compounds into the binding sites of the *S. aureus* TyrRS showed that the Schiff bases S1 and S2 gave the highest docking scores of -8.20 and -8.60 kcal/mol, respectively, and gave -7.98 and -8.44 kcal/mol, respectively, into the binding sites of the *C. albicans,* whereas the standard drugs, amoxicillin, and fluconazole showed binding affinity -7.56 and -6.11 kcal/mol for *S. aureus* and *C. albicans*, respectively. These results may explain the good antibacterial and antifungal activity of Schiff bases than azo compounds.

Compound S1 interacted with CYS37 of the largest pocket of 1JIJ protein with a distance of 3.00 Å and interacted with TYR47 of 5V5Z as H-acceptor interaction with distance of 2.74 Å. The compound S2 interacted with 1JIJ protein in the site of amino acid ASP195 as H-donor, while interacted with amino acids LEU15 and MET35 of 5V5Z protein as H-pi with distances 3.84 and 4.14 Å, respectively.



A1



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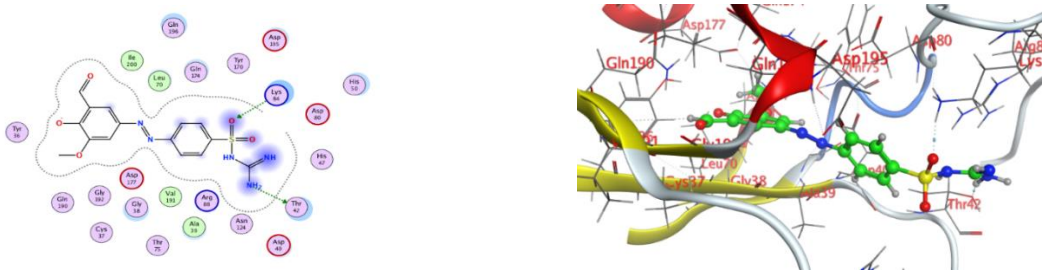

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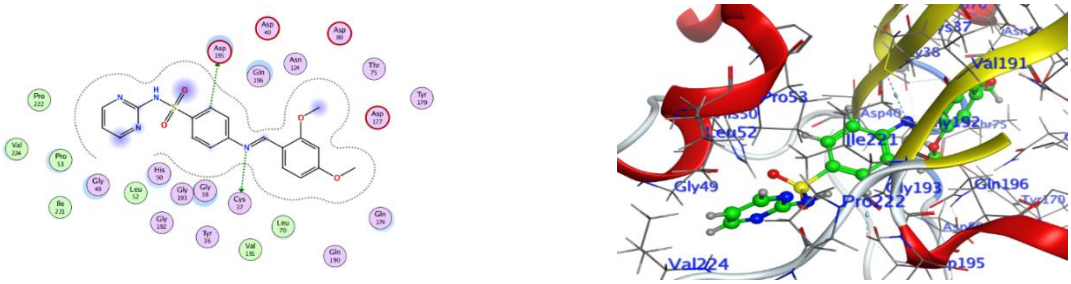
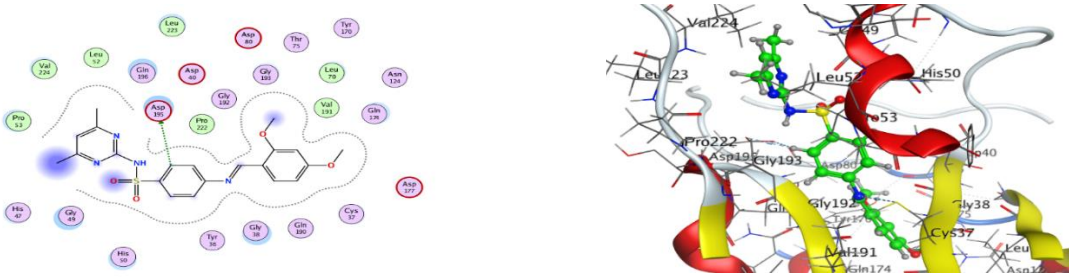


S1



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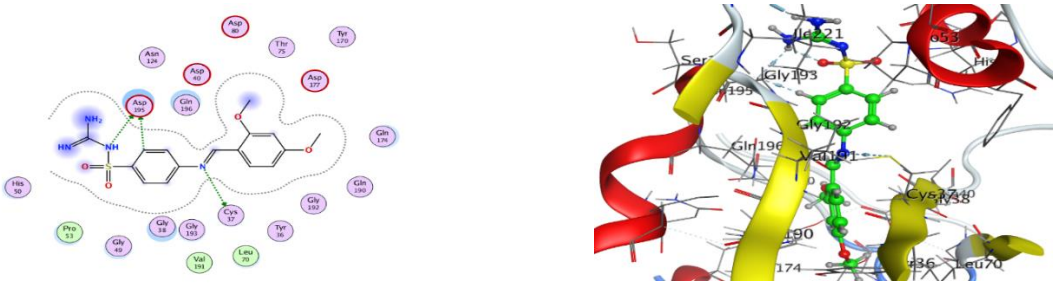


S3

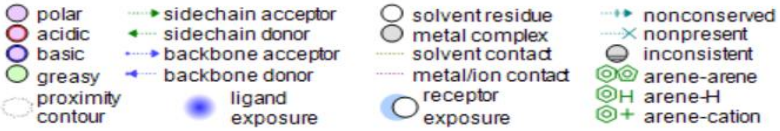
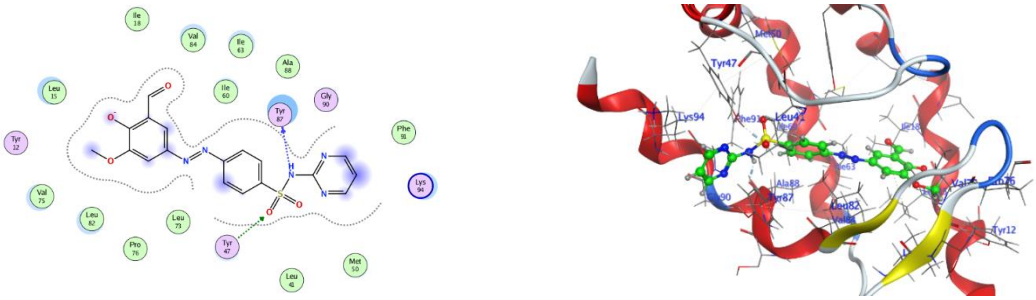
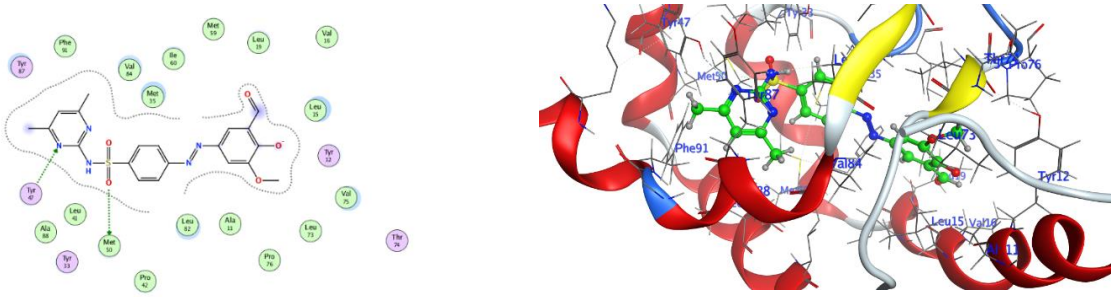


Figure 1: 2D and 3D binding affinity of six compounds with 1JIJ



A1



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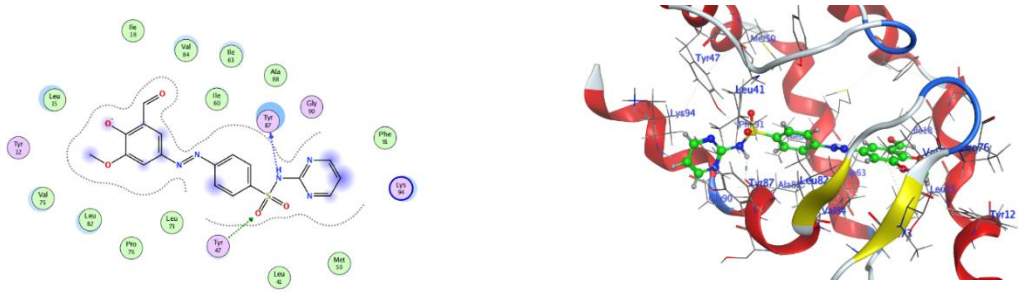

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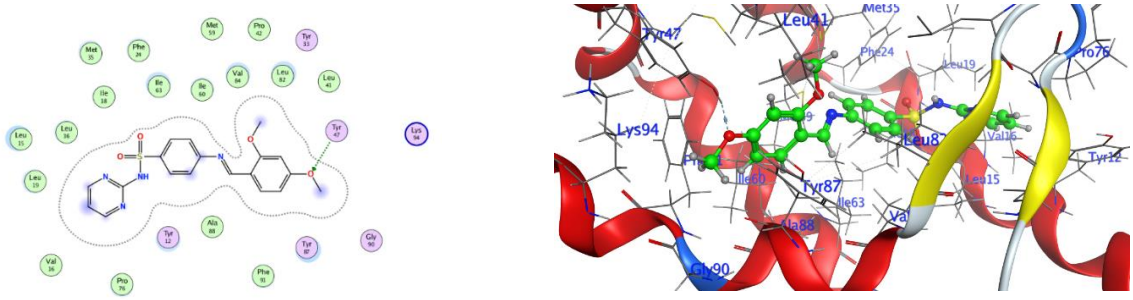
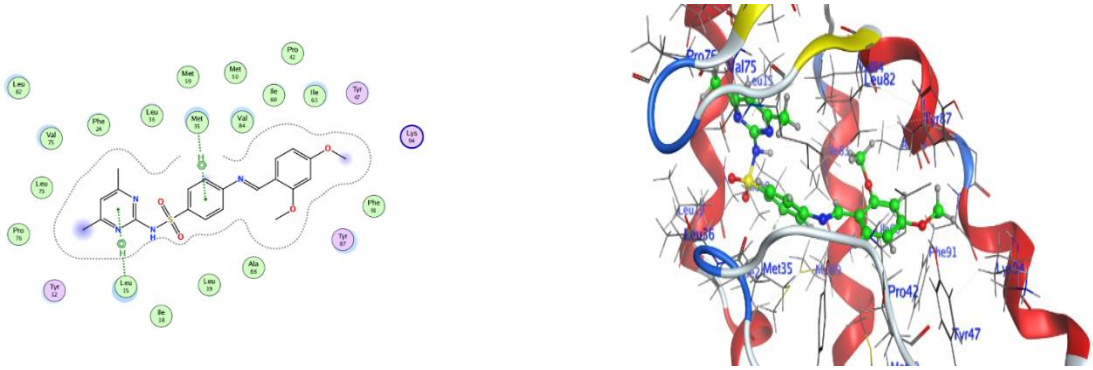


S1



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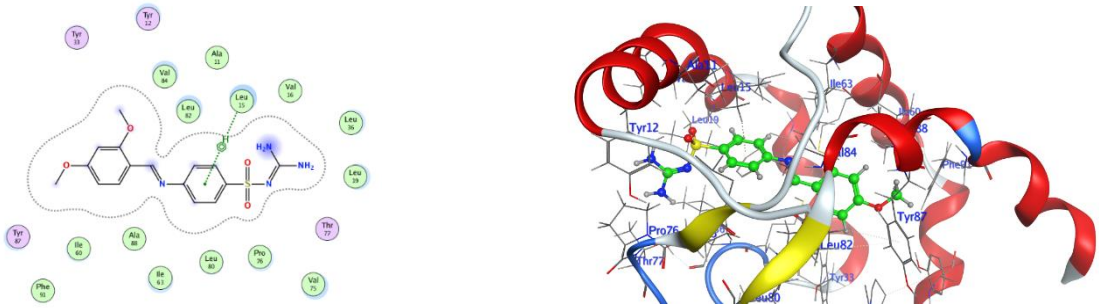

S3

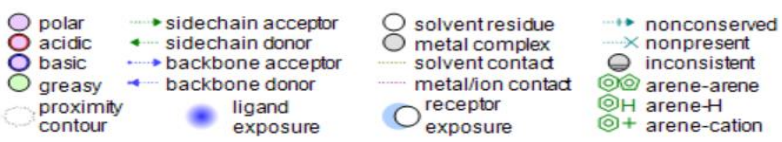

Figure 2: 2D and 3D binding affinity of six compounds with 5V5Z

Conclusion

The present study describes the synthesis of two series of azo compounds and Schiff bases. The results of antimicrobial evaluation revealed that especially compounds of Schiff bases (S1, S2, and S3) exhibited good activity against all microorganisms of bacteria and fungi. Whereas, A2 compound gave the best activity against *S. aureus* bacteria. In addition, docking studies of the synthesized compounds gave good affinity with the site of protein as compared with the standard drugs.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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