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Schiff Base Synthesis, Characterization, and Bioactivity

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Abstract: This study gives a summary of the investigation that was done. With the help of the generic formula R1N=CHR2, three new groups of physiologically active amino-substituted Schiff bases have been discovered. R1, which stands for 2-amino-benzothiazole, 4-amino-salicylic acid, and 4-aminophenol, is the group of substances employed in this investigation. Three different amino-substituted compounds and substituted aldehydes reacted in an ethanol solvent to produce R2 = 4-chlorobenzaldehyde, 2-chlorobenzaldehyde, salicylaldehyde, vanillin, and benzaldehyde. These compounds were described using various physicochemical techniques, including melting point analysis, elemental analysis, and multinuclear NMR spectroscopy (particularly 1H and 13C). Through screening against bacteria, fungi, and yeast, the in vitro biological activity of the free ligands and the metal complexes they form have been assessed. The metal complexes are more effective when comparing the metal complexes to Schiff base ligands. The main goal of this work is to create and assess Schiff bases produced from benzothiazole and aminophenol molecules. Investigating their potential as antibacterial and antifungal agents is the goal.

INTRODUCTION

Schiff bases are produced by the condensation reaction between primary amines and carbonyl compounds, first noted by Schiff in 1864. A common structural feature among the compounds under study is an azomethine group. The general formula RHC=N-R1, where R and R1 stand for alkyl, aryl, cycloalkyl, or heterocyclic groups, can be used to describe this group. It is essential to remember that these groups could contain various substituents. Alternative names for these substances include anils, imines, and azomethines. The relevance of a single pair of electrons in a nitrogen atom's sp2 hybridized orbital inside the azomethine group has been shown in numerous investigations. Schiff bases are well known for being great chelating agents because of their beneficial traits, including simplicity in manufacture, synthetic adaptability, and the peculiar properties of the C=N group. Notably, the ability of the azomethine group to form five- or six-membered rings with the metal ion is further enhanced when a functional group like -OH or -SH is close by. The literature is replete with documentation of this. The adaptability of Schiff base ligands is highlighted by their wide range of applications in various domains, such as biology, analysis, and industry, which calls for additional investigation and study in this area.

Hugo Schiff initially outlined the procedure of condensing primary amines with carbonyl compounds in 1864, when Schiff bases first appeared. The field of scientific research on Schiff base coordination chemistry has grown and expanded significantly in the modern era. In bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis, material science, separation and encapsulation processes, and the creation of molecules with unusual properties and structures, the significance of Schiff base complexes has been widely acknowledged and thoroughly investigated.

Due to their capacity to serve as bidentate ligands for transition metal ions, researchers first became interested in Schiff bases generated from aromatic aldehydes with an ortho-hydroxyl group. The azomethines made from salicylaldehydes had the most favorable correlation, according to later studies about the quantitative relationship between the structure and anticancer activity of a group of Schiff bases that were synthesized from aromatic amines and aldehydes with various substituents. The use of salicylaldehyde-derived Schiff bases as plant growth regulators, as well as their antibacterial and antimycoticeffects, have been documented in the literature. Previous studies have demonstrated that Schiff bases have some analytical applications. When describing the mechanisms of transamination and insemination reactions in biological systems, Schiff bases exhibit the unique -N=CH- (imine) functional group. In addition to Candida Albicans, Escherichia coli, Staphylococcus aureus, Bacillus polymer, Trichophyton gypsum, Mycobacteria, Erysiphe graminis, and Plasmopora viola, Schiff bases also have broad-spectrum activity against other species.

Due to their demonstrated exceptional selectivity, sensitivity, and stability towards specific metal ions, such as Ag(II), Al(III), Co(II), Cu(II), Gd(III), Hg(II), Ni(II), Pb(II), Y(III), and Zn(II), numerous different Schiff base ligands have been used as cation carriers in potentiometric sensors. Much research has been done on Schiff bases' catalytic properties. The cited work provides evidence of the catalysts' catalytic activity in the hydrogenation of olefins. There are several situations where biomimetic catalytic methods are appropriate.

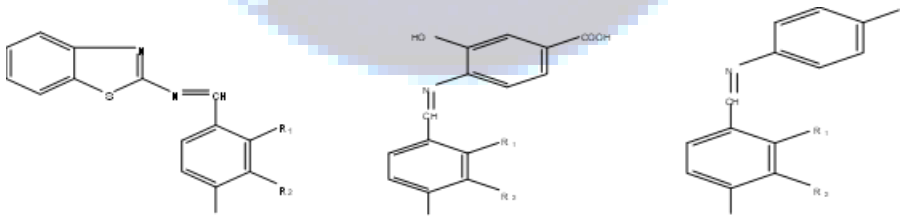
Schiff bases' propensity to spontaneously form a monolayer on the surface intended for protection has been discovered to make them particularly useful as corrosion inhibitors. They are effective in preventing corrosion because of this characteristic. A wide variety of amines or aldehydes are frequently used as commercial inhibitors. The existence of Schiff bases, most likely due to the C=N connection, is thought to be the cause of the increased efficacy shown in multiple instances. Chemisorption is the primary mode of interaction between the inhibitor and the metal surface. The inhibitor molecule must have active sites capable of transferring electrons to form chemical bonds with the metal surface. In situations of this kind, the inhibitor acts as a Lewis base, and the metal acts as an electrophile. The oxygen and nitrogen atoms, which serve as the protective compound's nucleophilic centers, have open electron pairs suitable for electron sharing. They collectively produce several absorption sites for the inhibitor and the benzene ring atoms, forming a stable monolayer.

Imines are also very important from a biological perspective. An imine linkage, a chemical connection between the protein opsin in the eye's retina and the aldehyde produced by vitamin A, is crucial to the physiological process of vision. The action of numerous enzymes, which are large proteins that assist chemical changes within cells, is greatly aided by vitamins known as coenzymes. The active form of vitamin B_6, pyridoxal phosphate, illustrates a physiologically important aldehyde. By establishing an imine bond with an amino acid, vitamin B_6 performs the role of a coenzyme by assisting in grouping enzymes. A transamination reaction, in which the amino group is moved from one amino acid to another, is made possible by the coenzyme attached to the enzyme. The metabolism and production of amino acids both depend heavily on this mechanism. The final step involves the hydrolysis of the imine, which is mediated by enzymes, producing pyridoxal and the altered amino acid.

Previous research has described the biological characteristics of Schiff bases, including their antibacterial and antifungal activities. Due to substantial research into their potential applications in anticancer and herbicidal actions, metal complexes have drawn much interest from the scientific community. These organisms serve as models for species that are crucial to human physiology.

Clinical features are displayed by O-phenylenediamine-derived Schiff bases. Isatin Schiff bases have antiviral, anti-HIV, antiprotozoal, and anthelmintic effects. These substances have noteworthy anticonvulsant efficacy and other pharmacological characteristics. Specific cobalt Schiff base complexes are effective antivirals0. It has been noted that Schiff bases made from 4-dimethylamine benzaldehyde have antimicrobial properties. Previous research has described the use of pharmaceuticals as antibodies and anti-inflammatory drugs.

The novel Schiff bases described here are produced via the acid-catalyzed condensation of aryl aldehydes with aromatic and heteroaromatic amines and show interesting biological characteristics. These substances might also be helpful ligands. Scheme 1 shows the structures of the Schiff bases made from 4-amino-salicylic acid, 4-aminophenol, and 2-amino-benzothiazole.



THE CURRENT STUDY USED A STANDARDIZED METHODOLOGY TO EXAMINE THE TOOLS AND RESEARCH TECHNIQUES.

Stuart Melting Point Apparatus SMP-3 uncorrected results were used to determine the melting points of Schiff bases. The Fisons EA 1108 CHNSO Micro analyzer was used for the elemental analysis. On a Bruker spectrometer, the internal standard was TMS, and DMSO was used as the solvent to acquire the lH and 13C NMR spectra.

Fluka provided the compounds used in the study, including 2-amino-benzothiazole, 4-amino-salicylic Acid, 4-aminophenol, 4-chloro-benzaldehyde, 2-chloro-benzaldehyde, salicylaldehyde, vanillin, and benzaldehyde. We bought the organic solvents from Merck.

Synthesis of 2-Amino-Benzothiazole-Derived Schiff Bases
In 25 milliliters of ethanol, 2 grams of 2-aminobenzothiazole was mixed with an equimolar amount of the corresponding aldehyde. After two hours of refluxing the resultant mixture, filtering separated the formed solid byproduct. The solid product was then purified via recrystallization from ethanol, washed with ethanol, and dried afterward.

The Synthesis of 4-Amino-Salicylic Acid-Derived Schiff Bases
In a solution containing 25 milliliters of ethanol, 2 grams of 4-amino-salicylic acid and an equivalent amount of the corresponding aldehyde were mixed. The resulting mixture underwent two hours of reflux; filtering extracted the formed solid product. The solid product was then cleaned using recrystallization with ethanol as the solvent. The finished product underwent additional ethanol treatment before being dried.

The Production of Schiff Bases from 4-Aminophenol
In a 25-milliliter ethanol solution, 2 grams of 4-aminophenol and an equivalent amount of each of the corresponding aldehydes were mixed. The resultant mixture was refluxed for two hours, and the formed solid product was isolated during filtration. Recrystallization procedures with ethanol then refined the isolated solid product as the solvent. The refined solid underwent additional ethanol washes of treatment before being dried.

Biological Activity In this section, we will discuss the subject's biological activity.
To determine whether the synthesized Schiff bases had any possible antibacterial and antifungal qualities, screening was done on them.

Assessment of Antibacterial
the bacterial B. subtilis strains. delicate, S. aeruginosa and E. coli were obtained from the University of Malaya's Department of Microbiology in Kuala Lumpur, Malaysia. The bacterial cultures were incubated at 30 0.1oC for 24 hours through the inoculation procedure onto nutrient agar. The Schiff bases were kept dry at room temperature and dissolved in 20 mg/ml dimethylsulfoxide (DMSO). The agar disc-diffusion method was used to evaluate each chemical's antibacterial abilities. At a temperature of 45°C, the Mueller Hinton Agar Media (15 cm3) solidified in Petri dishes. A standard saline solution containing a bacterial culture media with a concentration of 105–106 bacteria per ml was added to 50 L of Petri plates with a 9 cm diameter. After that, the plates were incubated. The discs were given the prepared Schiff bases (50 L) and forcefully pushed onto the solid agar medium. The Petri plates were incubated for 24 hours at 37 degrees Celsius. After the experiment, the inhibitory zones produced on the media were measured using a zone reader, with the measurements recorded in millimeters.

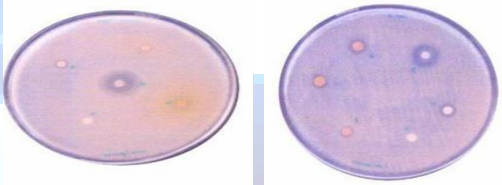


Figure 1. showing zone of inhabitation against subtilis, S. aureus, and E. coli

The zone of inhabitation regarding S. subtilis is shown in Figure 1. Aeruginosa and E. coli. Evaluation of Antifungal Activity
The pathogenic strains of Chalara corda and Aspergillus niger were purchased from the University of Malaya's Department of Microbiology in Kuala Lumpur, Malaysia. The Schiff bases were kept dry and at room temperature before being dissolved in dimethylsulfoxide (DMSO) at a concentration of 20 mg/mL. The agar disc-diffusion method was used to evaluate each drug's antifungal capabilities. At a temperature of 45°C, the Sabarod-prepared agar media (15 cm3) was added to the Petri dishes and left to set. In the experiment, prepared Schiff bases were impregnated with sterile filter paper discs measuring 10mm in diameter using a volume of 50L. Following their placement on the substrate, these discs were seeded with fungus. The plates were then incubated for one to seven days at a temperature of 27 degrees Celsius. After the experimental time, the inhibitory zones produced on the media were measured using a zone reader, with the measurements being recorded in millimeters.

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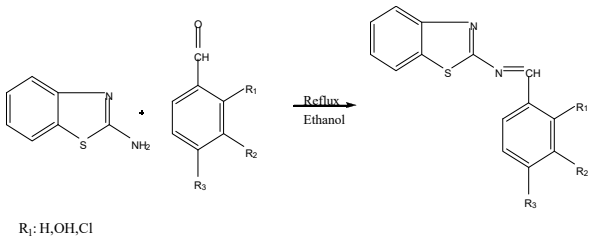
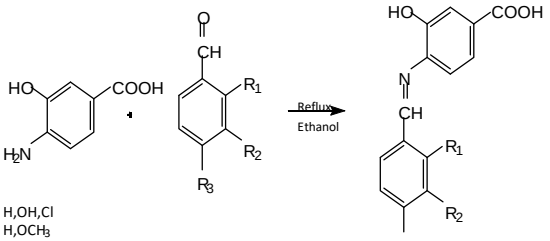


R_1: H,OH,Cl

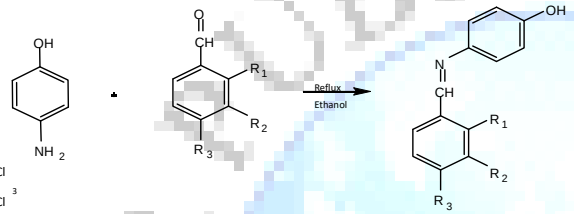
Scheme 2



R_1: H,OH,Cl
R_2: H,OCH_3

Scheme 3

Scheme 4



R_1: H,OH,Cl
R_2: H,OCH_3
R_3: H,OH,Cl

As shown in Schemes 2, 3, and 4, the condensation reaction between 2-amino-benzothiazole, 4-amino-salicylic acid, and 4-aminophenol with 4-chloro-benzaldehyde, 2-chloro-benzaldehyde, salicylaldehyde, vanillin, and benzaldehyde successfully produced a total of twelve unique Schiff bases. Table 1 lists and displays the analytical and physical data.

Comp no.	R_1	R_2	Molecular Formula	Melting Point °C	Yield (%)	Physical state	Solubility	Elemental Analysis: %C (Found)	%H (Found)	%N (Found)	%S (Found)
1	2-amino-benzthiazole	4-chloro-benzaldehyde	$C_{14}H_9ClN_2S$	125-126	63	Light yellow Solid	$CHCl_3$, C_2H_5OH, DMSO	61.65 (61.71)	3.30 (3.32)	10.27 (10.41)	11.74 (11.87)
2	2-amino-benzthiazole	2-chloro-benzaldehyde	$C_{14}H_9ClN_2S$	176-183	66	Light yellow Solid	$CHCl_3$, C_2H_5OH, DMSO	61.65 (62.03)	3.30 (3.41)	10.27 (10.63)	11.74 (11.82)
3	2-amino-benzthiazole	Salicylaldehyde	$C_{14}H_{10}N_2OS$	145-150	72	Orange Solid	$CHCl_3$, C_2H_5OH, DMSO	66.14 (65.94)	3.93 (4.15)	11.02 (11.45)	12.59 (12.61)
4	2-amino-benzthiazole	Benzaldehyde	$C_{14}H_{10}N_2S$	119-125	69	Light yellow Solid	$CHCl_3$, C_2H_5OH, DMSO	70.59 (71.46)	4.20 (4.41)	11.76 (11.89)	13.44 (13.51)
5	2-amino-benzthiazole	Vanillin	$C_{15}H_{12}N_2O_2S$	-	59	Yellow Liquid	$CHCl_3$, C_2H_5OH, DMSO	61.88 (63.89)	4.22 (4.47)	9.85 (9.97)	11.26 (11.78)
6	4-amino-salicylic acid	2-chloro-benzaldehyde	$C_{14}H_{11}ClNO_3$	140-223	61	Brown Solid	C_2H_5OH, DMSO	60.98 (61.21)	3.62 (3.71)	5.08 (5.21)	-
7	4-amino-salicylic acid	Salicylaldehyde	$C_{14}H_{12}NO_4$	181	74	Brick Red Solid	DMSO	63.36 (64.69)	4.28 (4.42)	5.44 (5.47)	-
8	4-aminophenol	4-chloro-benzaldehyde	$C_{13}H_{10}ClNO$	184-190	62	Brown Crystalline Solid	C_2H_5OH, DMSO	67.38 (67.36)	4.31 (4.30)	6.04 (6.01)	-
9	4-aminophenol	2-chloro-benzaldehyde	$C_{13}H_{10}ClNO$	150-159	67	Brown Crystalline Solid	C_2H_5OH, DMSO	67.38 (67.36)	4.31 (4.30)	6.04 (6.01)	-
10	4-aminophenol	Salicylaldehyde	$C_{13}H_{11}NO_2$	142-155	74	Reddish Crystalline Solid	C_2H_5OH, DMSO, $CHCl_3$	73.23 (73.22)	5.26 (5.15)	6.57 (6.55)	-
11	4-aminophenol	Vanillin	$C_{14}H_{13}ClNO_2$	208-215	57	Brown Crystalline Solid	C_2H_5OH, DMSO	69.13 (69.12)	5.34 (5.32)	5.76 (5.75)	-
12	4-aminophenol	Benzaldehyde	$C_{13}H_{11}NO$	187-192	64	Pale yellow Crystalline solid	C_2H_5OH, DMSO	79.18 (79.16)	5.58 (5.54)	7.10 (7.13)	-

Compound no.	Molecular Formula	OH (s)	CH (s)	C_6H_5 (m)	X (s)	X' (s)
1	$C_{14}H_9ClN_2S$	-	9.218	6.90-7.741 (8H)	-	-
2	$C_{14}H_9ClN_2S$	-	9.50	7.03-7.72 (8H)	-	-
3	$C_{14}H_{10}N_2OS$	9.4 (1H)	9.403	6.95-8.073 (8H)	-	-
4	$C_{14}H_{10}N_2S$	-	9.22	6.92-8.123 (9H)	-	-
5	$C_{15}H_{12}N_2O_2S$	9.58 (1H)	9.36	6.953-7.784 (7H)	3.435 (3H)	-
6	$C_{14}H_{10}ClNO_3$	9.04 (1H)	10.393	6.746-7.53 (7H)	-	10.85 (1H)
7	$C_{14}H_{11}NO_4$	8.97 (1H)	10.261	6.92-7.871 (7H)	8.97 (1H)	10.72 (1H)
8	$C_{13}H_{10}ClNO$	9.573 (1H)	8.616	6.791-7.916 (8H)	-	-
9	$C_{13}H_{10}ClNO$	9.549 (1H)	8.608	6.826-7.920 (8H)	-	-
10	$C_{12}H_{11}NO_2$	9.700 (1H)	8.888	6.848-7.596 (8H)	9.700 (1H)	-
11	$C_{14}H_{12}ClNO_2$	9.612 (1H)	8.428	6.755-7.494 (9H)	9.700 (1H)	3.838 (1H)
12	$C_{13}H_{11}NO$	9.60 (1H)	8.112	6.839-7.510 (9H)	-	-

[a] In DMSO at 295 K.
[b] X= OCH_3 for (compound 5), OH for (compound 7,10 & 11).
[c] X'=COOH for (compound 6 & 7), OCH_3 for (compound 11).
[d] Multiplicity is given as s = singlet, m = multiplet.
[e] Chemical shifts in ppm.

NMR (NUCLEAR ET AL.) SPECTROSCOPY

Table 2 displays the nuclear magnetic resonance (NMR) spectral information of the chemicals created in a desaturated dimethyl sulfoxide (DMSO) solution. The proton resonance assignment is made by examining their integration and multiplicity pattern. The Schiff bases' 1H nuclear magnetic resonance (NMR) spectra in dimethyl sulfoxide (DMSO) show different signals for compounds 1, 2, 3, 4, and 5 at chemical shifts of 9.218, 9.50, 9.4, 9.22, and 9.36 ppm, respectively. The protons connected to each compound's carbon-nitrogen double bond (CH=N) are given these signs. Numerous signals due to the aromatic protons in both rings are present in the range of 6.89-8.1ppm. The existence of the -CH=N- functional group in compounds 6 and 7, respectively, can be explained by the different signals at 10.39 and 10.26 parts per million (ppm) in the 1H nuclear magnetic resonance (NMR) spectra of

the Schiff bases produced from salicylic acid. Salicylic acid's hydroxyl (OH) group showed chemical shifts at 9.04 and 8.97 ppm. A singlet in offset at high values is a persistent feature of the phenolic protons, indicating that they are part of an intramolecular hydrogen bond with the nearby nitrogen atom. Unbound and unaffected by nearby atoms, the NH2 protons often produce a broad singlet peak in the 4-6 ppm range. The lack of this signal in the Schiff base spectra suggests that Schiff base manufacturing is taking place.

Carbon nuclear magnetic resonance spectroscopy (C NMR) is the subject of interest in academic studies.
Using 13C NMR spectra strengthens the support for the structural elucidation of the Schiff bases. For compounds 1 through 12, the 13C nuclear magnetic resonance (NMR) spectra have been recorded and are shown in Table 3. The presence of magnetically different carbon atoms, found by matching them to values published in existing literature, causes the number of signals detected. By contrasting the chemical shifts produced from experimental data with those derived from the incremental approach, the aromatic carbon in the structures of Schiff bases was identified. The Schiff bases' 13 C-NMR spectrum data agree with the proposed structures.

Antibacterial Efficacy Evaluation
The results of the antibacterial screening of the Schiff bases were achieved against all pathogens at a concentration of 20 mg/ml. According to Table 4, the inhibitory zones were measured in millimeters. The antimicrobial screening results show that Schiff bases are more effective than Aspergillus niger and Chalara corda against Staphylococcus aureus, Escherichia coli, and Bacillus subtilis. In addition, compounds 1 and 2 were found to have increased activity against all of the tested bacterial strains. This was due to the inclusion of an antimicrobial chloro group in the aldehydic moiety.

The antibacterial activity of the compounds is growing. The area of inhibited development grows in proportion to the increase in concentration.

The level of antifungal
According to the results of the evaluation of antifungal activity, the benzothiazole Schiff bases are more effective against all of the tested fungi than the salicylic Acid Schiff bases. Among all the tested fungi, compounds 1, 2, 3, 4, and 5 have the highest potency. The benzothiazole moiety is probably responsible for these compounds' increased activity. Compound 7 is more effective against all tested fungal strains than recognized pharmacological medicines. The anti-Aspergillus niger activity of compound 6 is noteworthy. Table 5 lists the outcomes of the antifungal activity.

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Compd. no.	1	2	3	4	5	6	7	8	9	10	11	12
CH	118.20	118.20	117.42	118.21	118.19	105.87	106.69	156.99	157.56	160.63	157.55	157.38
X	-	-	-	-	55.63	167.35	172.11	-	-	-	55.97	-
X'	151.76	152.55	151.69	152.40	152.63	-	-	-	-	-	-	-
R_1: C_1	128.73	128.37	125.95	128.80	124.87	131.17	129.66	156.20	156.80	157.45	156.14	152.88
C_2	125.80	125.89	125.74	125.72	125.79	121.24	119.93	116.19	116.20	116.43	115.77	116.33
C_3	128.73	128.37	125.95	128.80	124.87	158.53	156.29	130.28	131.29	132.66	128.78	130.34
C_4	131.09	131.07	131.07	128.96	131.04	121.09	119.78	123.10	122.97	119.42	119.53	123.19
C_5	129.81	129.61	129.72	130.60	129.68	134.21	134.43	130.28	131.29	132.66	128.78	130.34
C_6	130.96	130.97	131.07	128.96	131.09	136.53	136.87	116.19	116.20	116.43	115.77	116.33
R_2: C_7	127.17	127.92	119.93	128.60	118.16	116.29	117.16	123.10	128.72	116.93	122.60	128.44
C_8	129.14	133.49	155.64	127.20	127.76	132.86	155.15	129.32	136.92	157.45	123.97	128.44
C_9	128.80	127.92	119.93	127.14	154.91	116.29	117.16	129.32	128.72	116.93	116.11	128.44
C_{10}	134.71	130.44	131.60	126.98	148.93	129.87	131.95	135.78	129.19	132.95	143.63	128.44
C_{11}	128.80	127.33	120.30	127.14	131.82	127.66	122.74	129.32	143.06	119.42	150.11	128.44
C_{12}	129.14	130.44	131.60	127.20	127.76	129.87	131.95	129.32	129.19	132.95	115.77	128.44

[a] In DMSO at 295 K.
[b] X = OCH_3 for (compound 5 & 11), COOH for (compound 6 and 7).
[c] X' = C_2 of Thiazole ring.
[d] Chemical shifts in ppm.

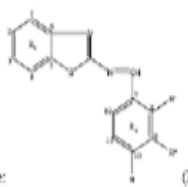
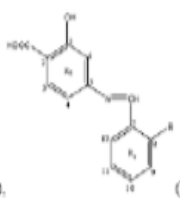
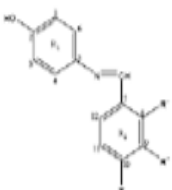
[e] R_1 and R_2 are:  (For Comp 1-5),  (For comp 6,7) and  (For comp 8-12).

TABLE IV. ANTIBACTERIAL ACTIVITY DATA[A-C] OF COMPOUNDS WITH GENERAL FORMULA: $R_1N=CHR_2$ (*IN VITRO*)

Compound no.	Zone of inhibition of sample (mm) in:		
	Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*
1	34	21	30
2	42	31.5	49
3	39.5	20	36
4	22	16	30
5	35	22	32
6	23	31	-
7	22	18	21
8	26	35	30
9	26	24	36
10	24	31	49
11	17	31	37
12	20	24	30
Std. drug (Amoxycillin)	18	12	14

[a] Concentration of sample = 20mg / mL of DMSO.
[b] Concentration of standard drug (Amoxycillin) = 10µg / disc.
[c] (-) = No activity.

TABLE V. ANTIFUNGAL ACTIVITY DATA[A-D] OF COMPOUNDS WITH GENERAL FORMULA: $R_1N=CHR_2$ (*IN VITRO*)

Compound no.	Zone of inhibition of sample (mm) in:	
	Aspergillus niger	*Chalara corda*
1	47	37
2	45	33
3	41	32
4	40	32.5
5	42	34
6	22	-
7	28	28
8	31	28
9	36	37
10	27	34
11	32	38
12	33	25
Std. drug (Ciprofloxacin)	20	12

[a] Concentration of sample = 20mg / mL of DMSO.
[b] Concentration of standard drug (Ciprofloxacin) = 10µg / disc.
[c] Incubation temperature (period) = 27 ± 1°C (7 days).
[d] (-) = No activity.

CONCLUSION

Analytical and spectroscopic methods synthesized and characterized Schiff bases made from 2-amino-benzothiazole, 4-amino-salicylic acid, and 4-aminophenol. The substances have shown notable effectiveness against all of the tested microorganisms

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