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Synthesis, pharmacological and modeling study of new sulphathiazole derivative

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ABSTRACT

Condensation of 4-amino-*N*-(1,3-thiazol-2-yl) benzenesulfonamide (sulphathiazole drug) with 3,4-dihydroxy benzaldehyde afforded Schiff base derivative in good yield. The new compound was characterized by elemental analysis, IR, ^{1}H, ^{13}C, and 2D (HSQC and HMBC- NMR) spectroscopy. It was screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus, Streptococcus spp, Klebsella spp, Salmonella spp, proteus spp* and *Pseudomonas spp* as well as fungicidal activity against *Aspergillus multi, Aspergillus niger, Candida albicans, Candida trobicalis and Candida krusi*. It exhibited also low to moderate activity against *Bacillus cereus, Salmonella spp* and *Psedumonas spp* and good active against *Aspergillus multi, Aspergillus niger, Candida albicans* and *Candida krusi*. The toxicity of the compound was also assayed by the determination of its LD_{50} value by using Dixon's up and down method, which exhibited an LD_{50} of 418.6 mg / kg of body weight. The *in silico* molecular modeling study of the synthesized Schiff's base was studied.

Keywords: Sulphathiazole, 3,4-Dihydroxy benzaldehyde, 2D- NMR, Antimicrobial, molecular modeling

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الملخص باللغة العربية

لقد أعطت عملية تكثيف المركب الكيميائي (4-amino-*N*-(1,3-thiazol-2-yl)benzenesulfonamide) المعروف بعقار السلفاثيازول مع المركب الكيميائي (3,4-dihydroxy benzaldehyde) مشتقا جديدا لقاعدة شيف بحصيلة إنتاجية جيدة.

تم تشخيص المركب الجديد بواسطة التحليل العنصري الدقيق وأطياف الأشعة تحت الحمراء والرنين النووي المغناطيسي للبروتون والكربون-13 ، والرنين النووي ثنائي المحور، وجرى اختبار المركب المحضر كمضاد بكتيري ضد كل من: (*Staphylococcus aureus*، *Escherichia coli*، *Bacillus cereus*، *Streptococcus* ، *Klebsella* ، *salmonella spp*، *proteus spp* ، و *Psedumonas spp*).

كما جرى اختبار المركب كمضاد فطري ضد كل من: (*Aspergillus multi* ، *Aspergillus niger* ، *Candida albicans* ، *Candida trobicalis* ، و *Candida krusi*).

Sulfa drugs, developed in the 1930s, were the first medications effective against bacterial diseases. They appeared as the first "miracle drugs" at a time when death from bacterial infections such as pneumonia and blood poisoning were common (1). Moreover, sulfa drugs had attracted special attention for their therapeutic importance as they were used against a wide spectrum of bacterial ailments (2,3). Sulfathiazole is an organosulfur compound used as a short-acting sulfa drug. It is an organic compound. Formerly, it was a common oral and topical antimicrobial, until less toxic alternatives were discovered. However, It is still occasionally used, sometimes in combination with sulfabenzamide and sulfacetamide, and in aquariums. Figure (1) below shows Sulfathiazole chemical structure.

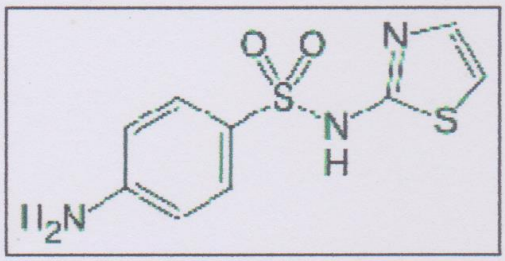


Figure (1): Chemical structure of sulphathiazole drug

Sulfa Schiff bases have been subjected to thorough studies, where a wide diversity of these derivatives were prepared and used in various biological and pharmacological fields (4-6). Schiff base compounds, which contain the azomethine (imine) group (–RC=N–) are usually prepared by the condensation of a primary amine with an active carbonyl compound (7). Schiff bases derived from sulfa drug and aromatic and hetero aromatic aldehydes are the most studied sulfonamide derivatives. These type of derivatives are very important because of their varied structures and biological activities (8-12). The Schiff bases are also known as anticancer and antiviral agents (13). The condensation of sulfa drugs with aldehyde gives biologically active Schiff bases. Keeping in view of the pronounced biological activity of the Schiff bases derived from sulfa drug, the aim of current study was to synthesize, characterize and investigate the antimicrobial ability and toxigenicity of Schiff bases derived from 3,4-dihydroxy benzaldehyde with sulfathiazole drug.

MATERIALS AND METHODS

Infrared spectra (IR) was recorded as KBr discs in the range of 4000-400 cm^{-1}using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 at the department of Chemistry College of

HMBC NMR spectra) were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz University, Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer. Melting points were measured by a Philip Harris melting point apparatus.

Antimicrobial activity:

The *in-vitro* biological screening of the 4-[(*E*)-(3,4-dihydroxybenzylidene)amino]-N-(1,3-thiazol-2-yl) benzenesulfonamide was investigated against various bacterial species: *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Streptococcus, Klebsella, salmonella spp, proteus spp* and *Psedumonas spp* and fungicidal activity against *Aspergillus multi, Aspergillusniger, Candida albicans, Candida trobicalis and Candida krusi* using the disc-agar diffusion technique (14). Muller Hinton agar was used as culture media for antibacterial activity. The antifungal activities were tested against selecte fungus by disk diffusion method. Recommended concentrations 50, 100 and 200 µg/ml of the test samples in DMSO solvent were introduced in the respective methods. Antibiotic drugs Gentamycin (10 mg) were used as control for bacteria and Flurazol (10 mg) for fungi, respectively. Petri plates containing 20 ml of Mueller Hinton Agar were used for all the bacteria tested. *Aspergillus multi, Aspergillus niger, Candida albicans, Candida trobicalis and Candida krusi* strains were cultivated in Sabouraud's dextrose agar. Sterile Whatman no.1 filter paper disks (6mm in diameter) impregnated with the solution in DMSO of the test were placed on the Petri plates. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. The plates were incubated for 24 hrs. in the for bacteria and 72 hrs. for fungi at 28 °C. The inhibition zone diameters were measured in millimetres using a calliper vernia.

Acute toxicity (LD_{50}):

Animals. All experiments were performed on 10-14-weak old male and female Balb/c mice weighing 22-25 gm at the time of treatment by using up-and-down method formed by Dixon(15).

Male and female mice were injected intraperitonially with different doses of the Sulphathiazole derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD_{50} were determined after reading final results (response-dead (X) or non response alive (O), then the following equation was applied:

$$LD_{50} = XF + Kd$$

The estimate of LD_{50} is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels. (d) is the tabulated value (table 1).

Table (1): Data represented Dixon values (15).

	K represented serial tests started with				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
	X	XX	XXX	XXXX	

Synthesis of Schiff base:

4-[(E)-(3,4-dihydroxybenzylidene) amino]-N-(1,3-thiazol-2-yl) benzenesulfonamide (3):

A solution of 4-amino-*N*-(1,3-thiazol-2-yl)benzenesulfonamide (sulphathiazole)(1) (2.0 g, 7.83 mmol) in EtOH (25 ml) was added to a hot ethanolic solution of 3,4-dihydroxy benzaldehyde(2) (1.08g, 7.83 mmol) followed by addition of three drops of glacial acetic acid. The mixture was heated under reflux for 3 hrs. and then left at refrigerator overnight.

The solid was filtered and washed with acetone and the final product was recrystallized by $CHCl_3$-EtOH(4:1) to give 3 as a brown-dark crystals (79%), m.p.=137-140°C. FT-IR (KBr,cm^{-1}): 3466 (O-H), 3356(N-H), 3065 (C-H aromatic), 2900, 2810 (C-H aliphatic), 1668 (C=N), 1598 (C=C), 1192 (C-O). ^{1}H NMR (DMSO-d_6); δ9.71(s, 2H,OH); 8.38 (s,1H,CH=N) (7.80) m, 7H, Ar-H); 5.82 (s,2H, $H_{thiazole}$). ^{13}C NMR(DMSO-d_6); δ152-1112 (C-Ar), 162(C-CH=N), 168 (C-C=N). Analytical calculated for $C_{16}H_{13}N_3O_4S_2$ (375.4): C, 51.14; H, 3.46; N, 11.18. found: C, 50.94; H, 3.12; N, 11.4. (figure 2).

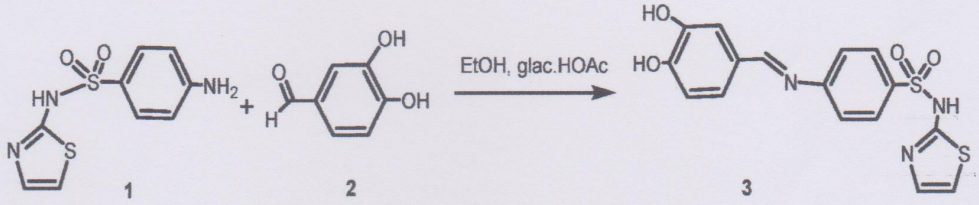


Figure (2): Preparation of new Schiff base 3 derived from sulphathiazole derivative 1

RESULTS AND DISCUSSION

Chemistry:

Isolated yield, melting point, color and spectral data IR and ^{1}H NMR of synthesized new compound 3 were reported. The present work describes the synthesis of new Schiff base derived from sulphathiazole and aldehyde to produce bioactive Schiff base. Thus, treatment of 4-amino-*N*-(1,3-thiazol-2-yl) benzenesulfonamide (sulphathiazole) with 3,4-dihydroxy benzaldehyde in 1:1 mole ratio gave the new organic compound in good yield. IR spectra for the synthesized compound displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The IR spectra of new prepared compound showed strong bands in the rang 3466-3356 cm^{-1} due to ν(O-H) and ν(N-H) respectively. The IR spectra of the synthesized compound showed bands at 1668, 1598 due to(C=N) and(C=C) cm^{-1} respectively (figure 3).

The ^{1}H NMR spectra of studied synthesized compound was recorded in DMSOd_6 solution and show all the expected protons with proper intensity ratio, It is worthy to note that the proton of Ar-OH resonate as a single at 9.7 ppm which is in agreement with previously reported data (16). The aromatic protons of the compound appeared within the range 7.80-6.75 ppm. The proton of azomethine (CH=N) resonate as a singlet at 8.38 ppm (figures 4 and 5). The ^{13}C NMR spectra of synthesized compound showed the expected resonance signals and is consistent with their structures. The large variation of carbon atoms bearing sulphar can be explained by the polarity of the C-S bond in thiazole ring (figure 6). HSQC and HMBC NMR showed the correlation of protons and carbon in aromatic rings which support the chemical structure of synthesized new compound (Figures 7, 8)

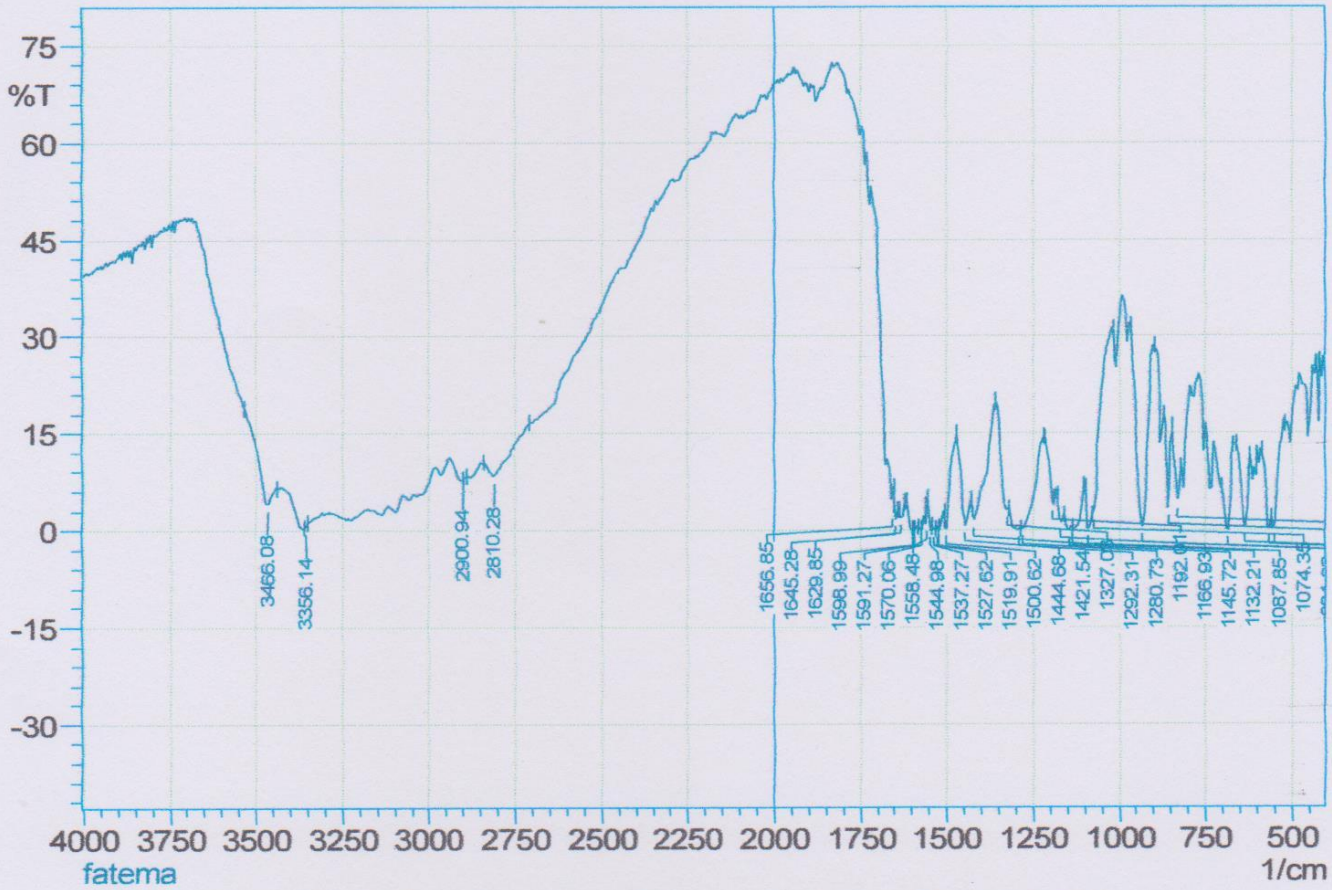
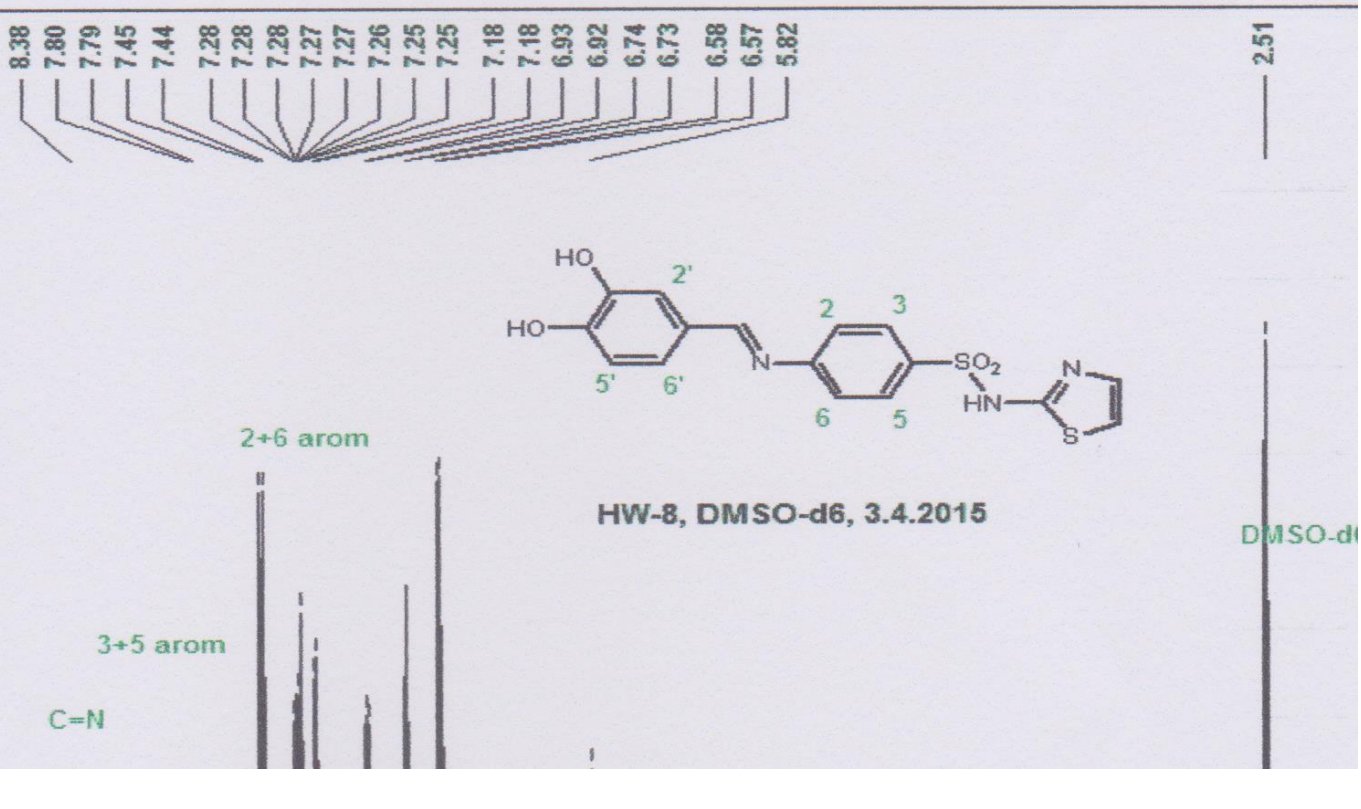


Figure (3): Infra red spectrum of the new derivative of suphathiazole



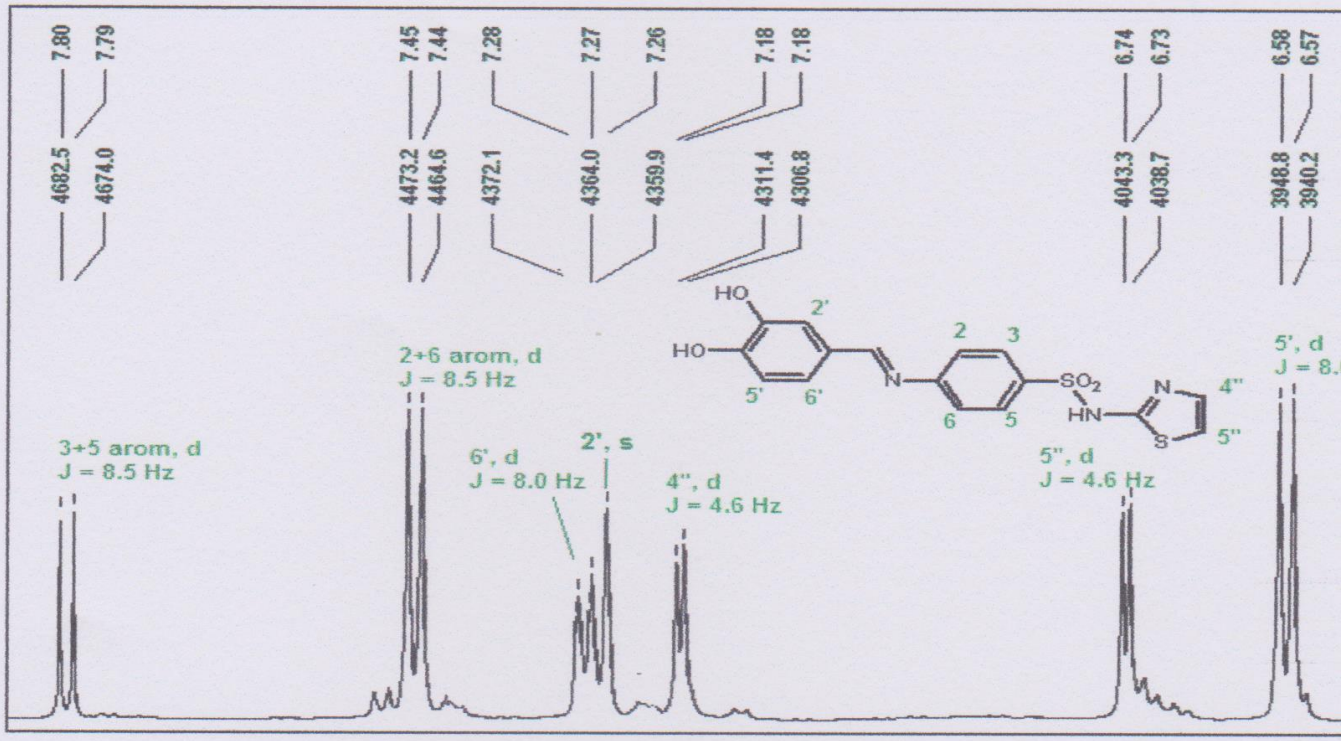
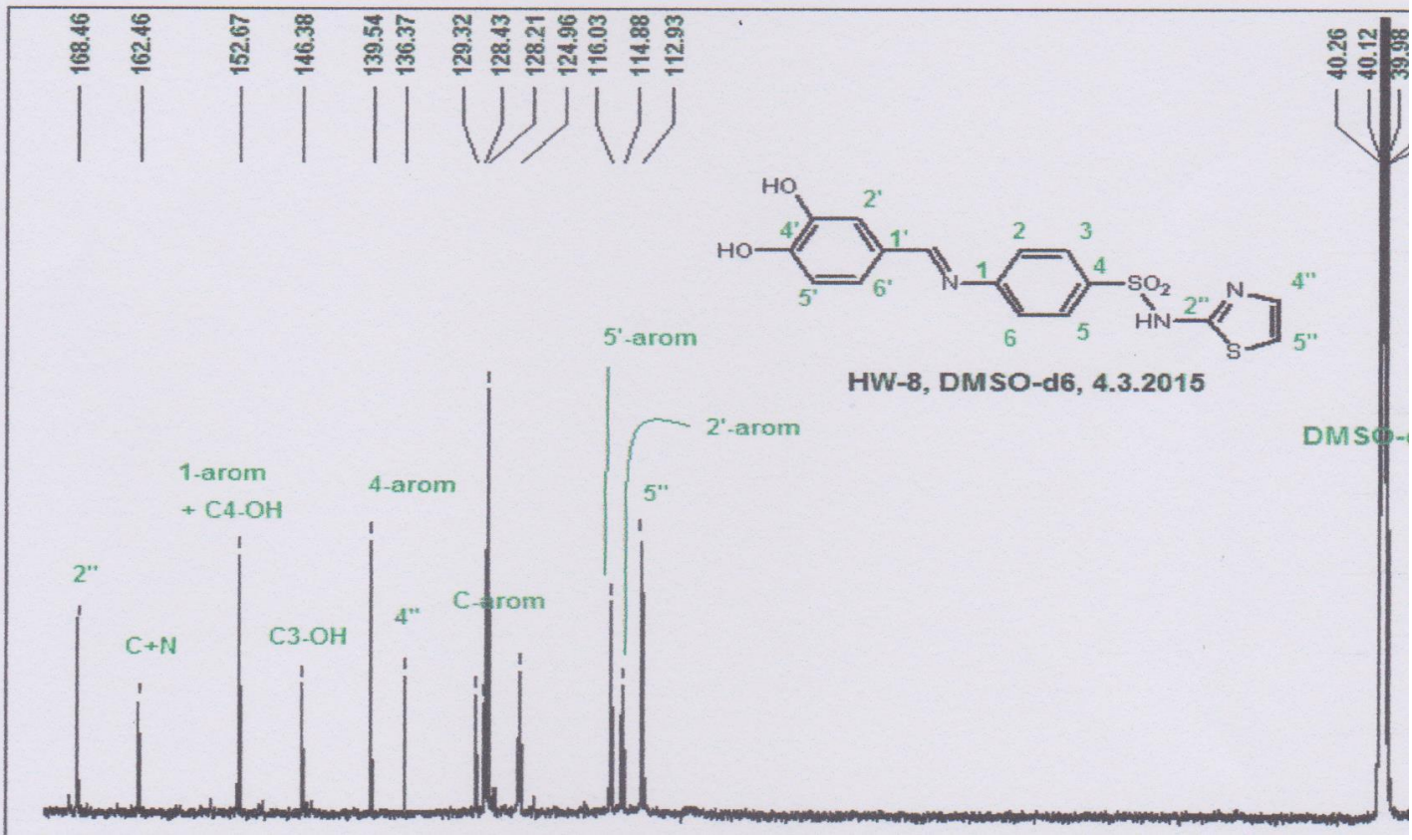


Figure (5): ^{1}H NMR expansion spectrum of the new derivative of suphathiazole



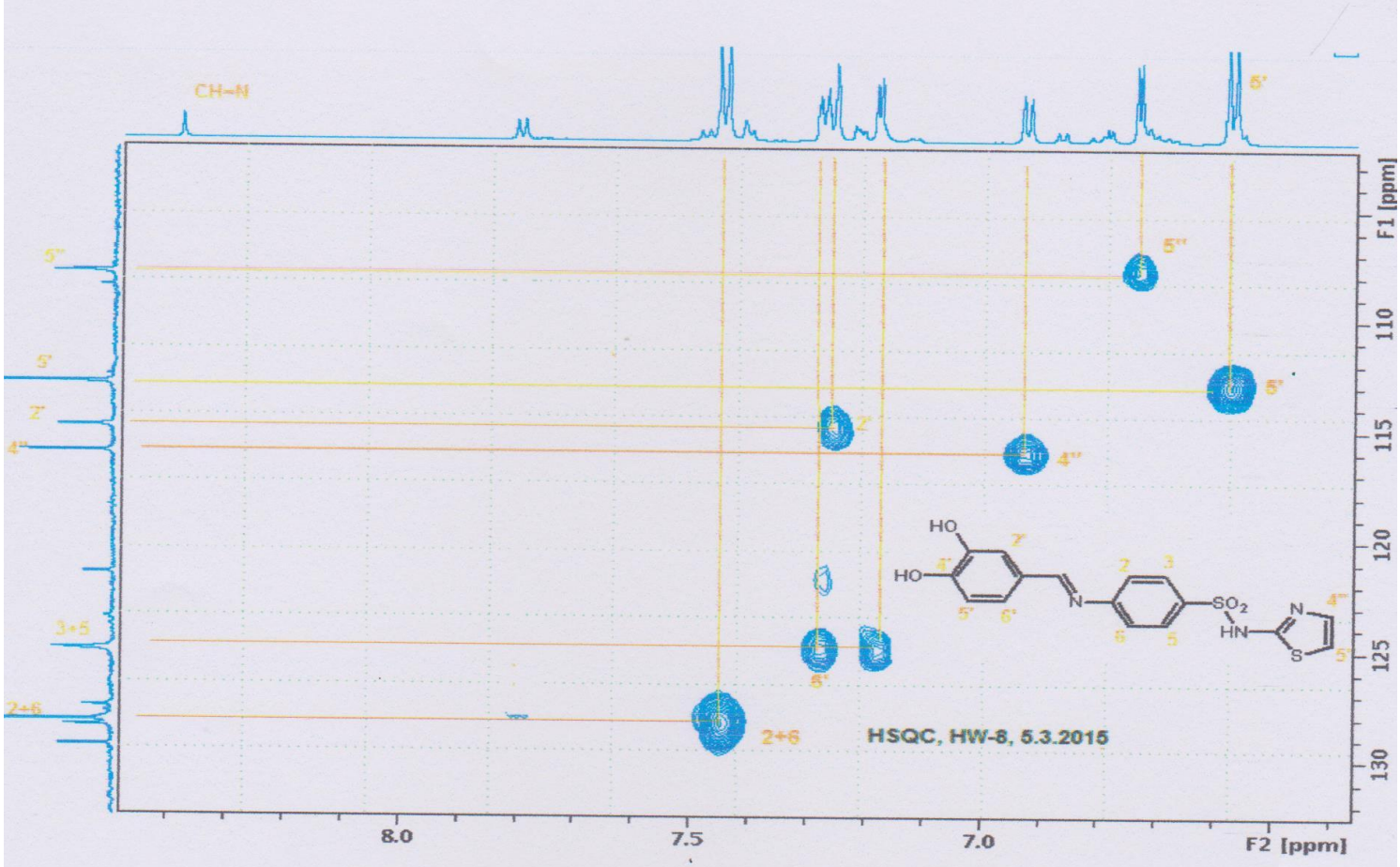
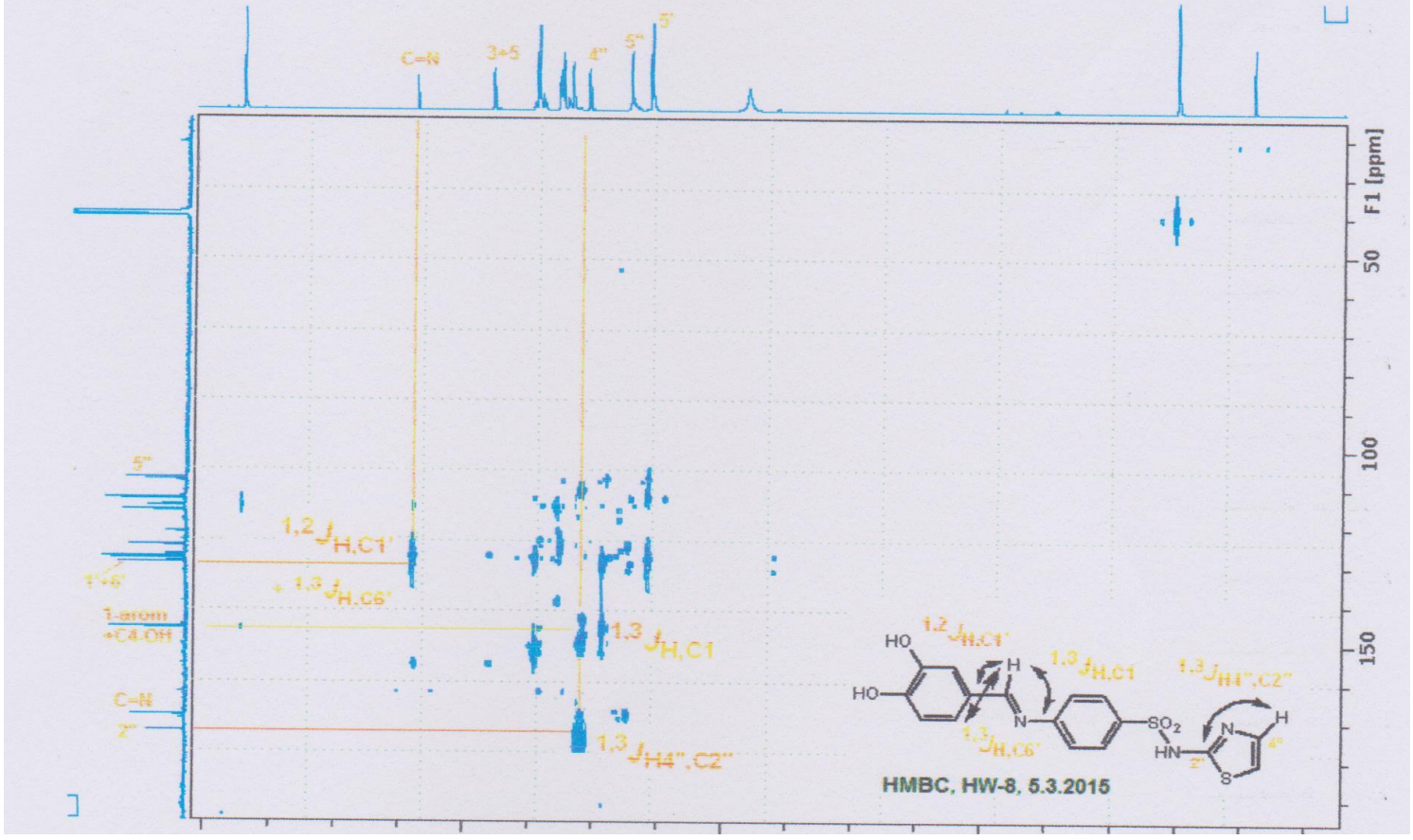


Figure (7): HSQC NMR spectrum of the new derivative of suphathiazole



Pharmacological study:

1. Median lethal dose (LD_{50}): Determination of the 50% of lethal dose (LD_{50}) of the studied compound *in vivo* was detected in the mice by using the "up-and-down" procedure described by (15). In the experiment we using 10 animals of white mice 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (250, 300, 350, 400 mg/k.gb.w) in 0.1 ml (dimethyl sulphoxide) DMSO, were administered and chosen with equal spacing (concentrations) between doses. Mortality was recorded after 24 hrs. that each one animal treated with one dose and after 24 hrs. was recorded as O if the animal lives and then increased the treated dose. While X recorded for the death of animal and then decreased the dose according for the result of the animal the code which formed as being (OOXX) and according for Dixon value was get and the LD_{50} was determined according to the formula employed by (15).

LD_{50} =Xf+ Kd
LD_{50} = 400 + 0.372 x 50
LD_{50} = 418.6 mg / kg b.w
1/10 LD_{50}= 41.86 mg / kg (1 kg = depending on the weight mice 25 g.
1/10 LD_{50}=1.0465 mg /mice depending weight mice 25 g

2. Antimicrobial study: The results antimicrobial activity are shown in table studied compound showed no activity *Staphylococcus aureus*, *Escherichia Streptococcus* ,*Klebsella spp,* and *proteus* low active in *Bacillus cereus* at 200 µg moderate activity in *Salmonella sp Psedumonas spp.* The results of antifungal of the compound showed no active towards *trobicalis* , but good active against *As multi, Aspergillus niger*, *Candida albic Candida krusi* compared with controls (tabl bacteria and fungi were supplied from depa Microbiology, College of Veterinary M University of Basrah.

Table (2): Microbial activities of the Schiff-base derivatives of sulphathiazole drug (Diameter of inhibition zon for different microbial species)

Microorganism	50µg/ml	100µg/ml	200µg/ml	Gentamycine (10 µg)	
E. coli	-	-	-	22	
S.aureus	-	-	-	22	
Sreptococcus	-	-	-	20	
Klebsella	-	-	-	22	
Bacillus	-	-	7	13	
Salmonella	-	9	9	25	
Psedumonas	-	-	9	22	
Proteus Spp	-	-	-	20	
Candida albicans	-	-	-	-	
Candida trobicalis	8	10	10	-	
Candida krusi	7	8	10	-	
Apergillusmulti	10	10	12	-	
Aspergillus niger	9	9	10	-	

Molecular modelling analysis: The molecular docking was performed by using SYBYL- X 1.1 and the docking result was shown by PyMol (17). Our molecular docking analysis of the new analogue 3 based on the modelling study, which was performed to understand the binding mode of this compound with the *Candida tropicalis* amino acids binding pocket (PDB code: 1N9G (18).
Compound 3 showed binding energy score -8.3, indicating as electivity of substituted thiazole-Schiff base analogue in its binding to the enzyme pocket (figure 9). As shown in figure (9), proton of C-3 of aromatic residue as well, in additio interaction between terminal NH_2 proton of with sulphur atom of the thiazole scaffold. non-bonded of Gly175, Met277, Asn1 Gly276 of *Candida tropicalis* amino acid were observed surrounded the synthesized m

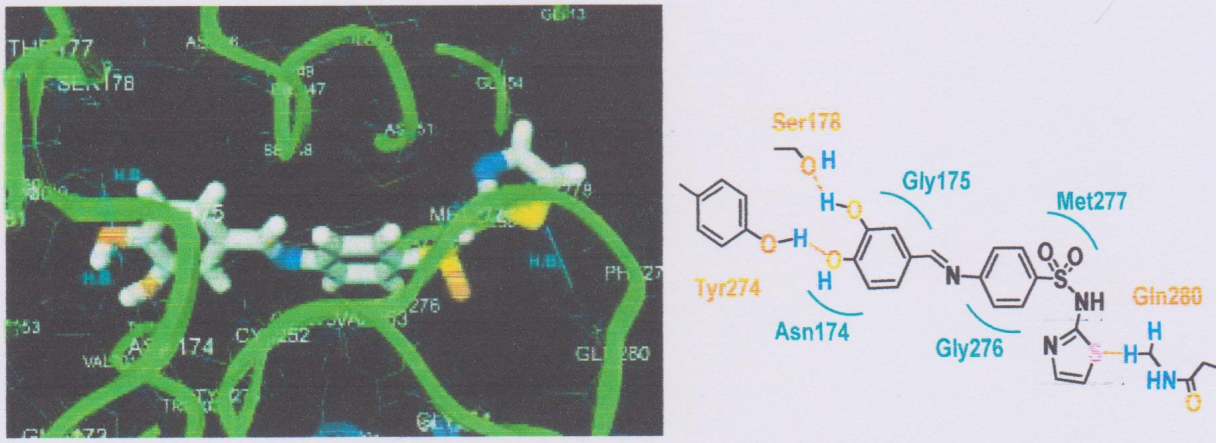


Figure (9): Docked conformation of 3 showing three hydrogen bonds: Proton of OH group of aromatic moiety of Tyr274 with *O* atom of OH proton at C-4 of aromatic ring of compound 3, Ser178 with *O* atom of OH group at C-3 of aromatic residue as well, in addition to the interaction between terminal NH2 proton of Gln280 with sulphur atom of the thiazole scaffold. Besides, non-bonded interaction of Gly175, Met277, Asn174 and Gly276 of *Candida tropicalis* amino acid residues were observed.

CONCLUSION

In conclusion, the present study reported the synthesis of new sulphathiazole analogue namely 4-[(*E*)-(3,4-dihydroxybenzylidene)amino]-*N*-(1,3-thiazol-2-yl)benzenesulfonamide, which revealed moderate *in vivo* toxic effects by LD_{50} measurement. In addition, the *in vitro* antibacterial and antifungal activities against some bacterial and fungi were studied, for further future biological studies.

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