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SYNTHESIS AND PHYSIOLOGICAL STUDY OF SCHIFF BASE DERIVED FROM SULFANILAMIDE AND VANILINE

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ABSTRACT

The design and production of innovative and safer drugs attracted to organic chemists is urgently required in order to synthesize new compounds with the potential of biological and chemotherapeutic activities. We're reporting here, the condensation of 4-aminobenzene-1-sulfonamide (sulphanilamide drug) with 4-Hydroxy-3-methoxy-benzaldehyde (vanillin), yielded derivative of Schiff base in good yield. Elemental analysis (CHN), IR, 1H and 13C-NMR spectroscopy were used to characterize the synthesized compound. Using the Balb/c mouse model, the toxicity of the synthesized compound was determined. The up and down method of Dixon was found to have a body weight of 1677.2 mg / kg LD50 and mild toxicity. The results showed the ability of the prepared compound to improve the TWBC and DWBC values approach to control, giving HSV a less harmful effect than the sulfanilamide drug.

INTRODUCTION

As the first drugs used against some bacterial infections, sulfa drugs are know n as treatments of eye infections, measles, meningitis, and other meningitis such as actinomycea, infections of the urinary tract as preventive and therapeutic compounds. (1-3).

Schiff bases are a significant class of organic compounds with biological activ ities and structural chemical significance, and because of promising antibacterial and antiviral activities, as well as metal chelating effects and other pharmacological effects, many Schiff base derivatives have been used in different physiological and coordination chemistry fields (4-6).

Schiff bases was prepared via indole-3-carbox aldehyde condensation with a number of sulfa medicinal products, including sulfanilamide, sulfapyridine, sulfadiazine, sulfamethoxazole, sulfamethoxypyri dazine and sodium sulfacetamide. (7)The goal of the present study is to synthesize and investigate the 4-aminobenzene-1-sulfonamide (sulphanilamide drug) and 4-hydroxy-3-methoxy benzaldehyde (vanillin) of the Schiff base and characterized by using physical and spectral techniques, including elemental analysis, IR and NMR. Acute toxicity and hematological Parameters of synthesized compound was performed.

Materials and methods

Instrumentation

The IR spectra were recorded at the Polymer Research Centre, University of Basrah, Basrah, Iraq, in the range of 4000-200 cm-1 on a Pye-Unicam SP3-300 spectrometer using KBr disks. The IR, 1H, and 13C-NMR spectra were measured at 600 MHz on the Bruker, with TMS as internal reference at the University of Konstanz, Germany. The melting point was determined at the College of Veterinary Medicine, University of Basrah, Iraq, using a Philip Harris melting point unit.

Acute toxicity (LD50) study

The lethal dose (50%) of the synthesized compound was calculated in Balb/c mice using the up and down method (8). After a series of studies, male and female mice aged 4-6 weeks were injected intraperitoneally with different doses of the synthesized compound. A series of tracks were carried out using this approach with equal spacing between doses: increased dose following a negative response and decreased dose following a positive response. Testing continued until the "nominal" sample size chosen was achieved. After reading the final result (response-dead (X) or non-response alive (O), LD50 was calculated according to the following equation:

LD50 = XF + Kd .

The LD50 approximation is XF + Kd where the final test level is XF and the distance between the dose levels is K. The tabulated value is d.

Synthesis

Synthesis of 4-{(E)-[(4-hydroxy-3-methoxyphenyl)methylidene]amino}benzene-1-sulfonamide.

4-aminobenzene-1-sulfonamide (sulphanilamide drug) (5.8 mmol, 1.0 g) in 25 methanol was added to hot ethanolic solution of 4-hydroxy-3-methoxy benzaldehyde (Vanilline) (5.8 mmol, 0.883 g), glacial acetic acid (3 drops) were added and the resultng solution was refluxed for 3 h and then lifted over-night. The solid product obtained was purified by washed with ethanol and the final product was recrystallized to create yellow crystals by using chloroform: methanol (8:2, v : v).

Yield: 83%.

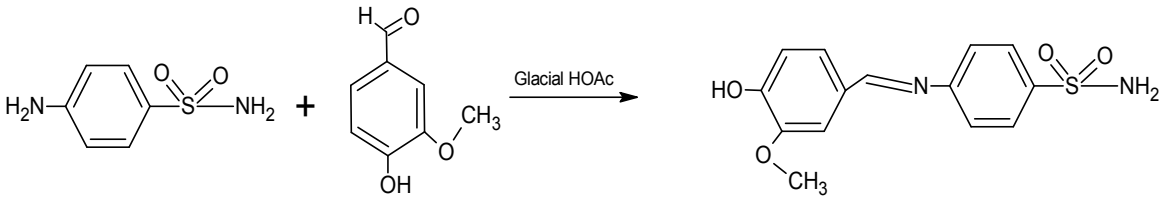
M.p.: 183-185 °C.

FT-IR (KBr, ν, cm^{-1}): 3484-3345 (NH), 3254 (OH), 3087 and 3013 (CH-Ar-H); 2965 (CH-Aliph.), 1670-1586 (C=C, C=N).

^{1}H NMR (600 MHz, DMSO-*6d*, δ, ppm): 3.84 (s, 3H, O-CH_3), 5.79 (d, 2H, NH_2), 7.84-6.58 (m, 7H, Ar-H), 8.47 (s, 1H, CH=N), 9.77 (s, 1H, OH).

^{13}C NMR (150 MHz, DMSO-*6d*, δ, ppm), 55.5 (1C, OCH_3), 112.4-154.8 (12C, C-Ar), 162.0 (1C, CH=N).

Anal. calcd. for $C_{14}H_{14}N_2O_4S$: C, 54.90; H, 4.57; N, 9.15. Found: C, 54.37; H, 4.16; N, 8.86%.



Scheme1:Synthesis of 4-{(E)-[(4-hydroxy-3-methoxyphenyl)methylidene]amino}benzene-1-sulfonamide

RESULTS AND DISCUSSION

Chemistry

Schiff base derived from sulfanilamide drug and vanillin was describes in this work via reaction of 4-aminobenzene-1-sulfonamide with 4-Hydroxy-3-methoxy-benzaldehyde in 1:1 ratio to yield imine compound, Scheme 1. In certain regions and characteristic bands in the fingerprint and other regions, IR spectra for the compound showed typical characteristics. The IR spectrum confirms the existence of the azomethine group (CH = N) extending about 1586 cm1 with a sharp area .

1H NMR spectrum of synthesized compound show single signal attributed to azomethine proton (CH=N) at δ 8.47 ppm, 1H NMR spectrum of Schiff base show a doublet at δ 5.79 ppm due to NH2 protons and singlet at 3.84 ppm due to methoxy group OCH3. The region at δ 6.58-7.84 ppm due to aromatic protons. 1H NMR spectra of synthesized compound show singlet at δ 9.77 ppm due to phenolic OH (8).

The 13C NMR spectrum of synthesized compound was measured in DMSOd6. The spectra revealed the presence of –CH=N group around δ 162.0 ppm. The signals at the range δ 110.5-154.8 ppm due to C-Ar. These spectra data sports the structure of synthesized compound (9).

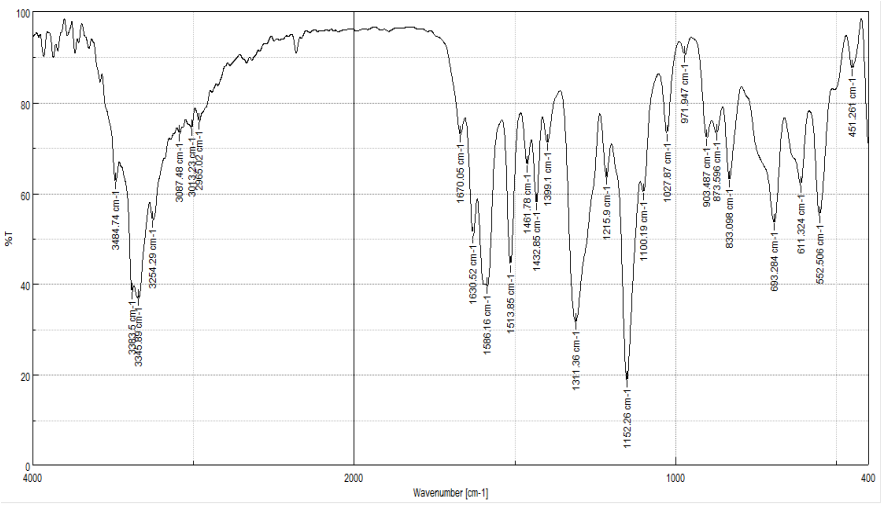


Figure 1: Infra-red of synthesized compound.

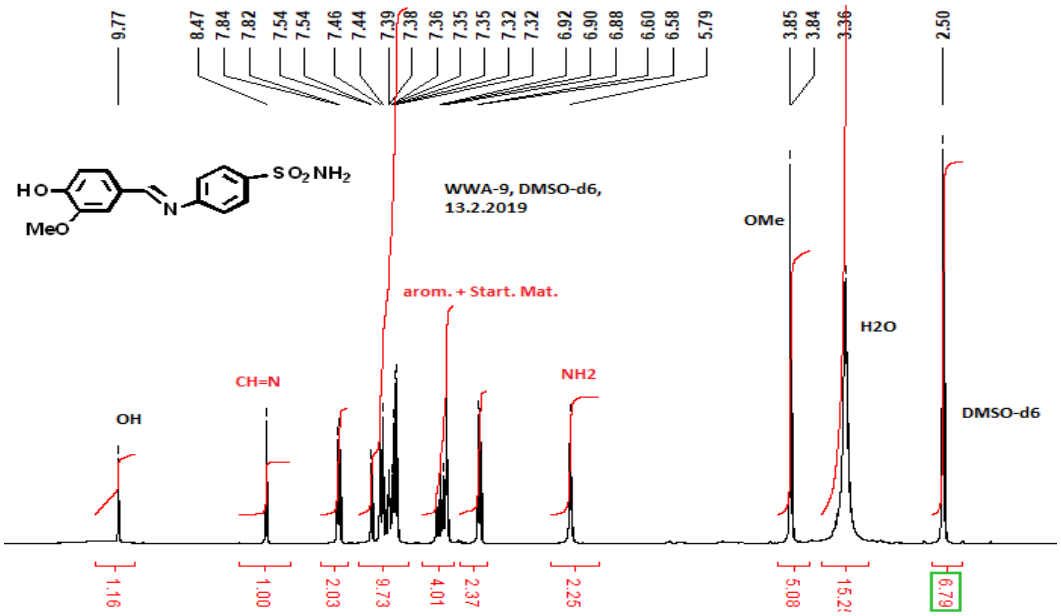


Figure 2. 1H NMR of synthesized compound.

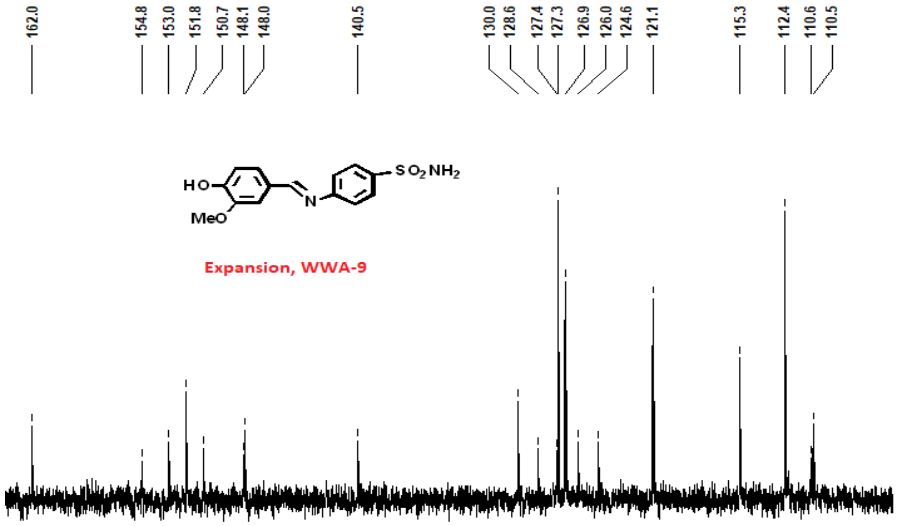


Figure 3. 13C NMR of synthesized compound.

Physiological Study:

Determination of the 50% of lethal dose (LD50) of the synthesized Schiff base *in-vivo*

The LD50 of the synthesized compound was detected in mice using the "up-and down" technique defined by Dixon (10), in the experiment using 10 white mice animals 7-8 weeks of age. Graded doses of injection were administered to each animal, a series of concentrations (250,300,350---1600 mg / kg.bw) in 0.1 mL of dimethyl sulfoxide (DMSO) were administered and selected with equal spacing (concentrations).

Mortality was reported after 24 h that each animal received a single dose, and after 24 h that each animal received an O if the animal survived and then increased the dose treated. While X reported the death of the animal and then reduced the dose according to the outcome of the animal, the code established as being (OOOX) and Dixon value was obtained and the LD50 was calculated accordingto the formula used by Dixon: (LD50 = Xf + Kd; LD50 = 1600 + 1.544 × 50 = 1677.2 mg/kg.bw).

White blood cells study

Animals

30 local male rabbits aged 6 months and weighing 1000-1600 grams were used and brought to Basra town from the local market. Animals were housed in regular laboratory-controlled cages at temperatures of 25 ± 2 ° C and 12 hrs. Regular delivery of light / dark cycle, food and water ad libitum. In the animal house where experiments were carried out, one week after acclimatization.

Experimental design

Rabbits are divided into 5 groups (6 rabbits in each group) as follows:-

Group I (control): 6 male rabbits were administrated 0.5 ml dimethyl sulphoxide (DMSO) intrapertoineal for three weeks daily.

Group 2 (Treated 1) T1: 1/20 0f LD50 (195 mg / kg) of 0.5 ml dissolved sulfanilamide drug (DMSO) intrapertoineal was administered to 6 male rabbits for three weeks daily.

Group 3 (Treated 2) T2: 6 male rabbits got 1/20 0f LD50 (195 mg / kg) of 0.5ml (DMSO) intrapertoineal dissolved sulfanilamide drug and 0.15 ml / kg of vitamin E orally administered for three weeks daily.

Group 4 (Treated 3) T3: 1/20 0f LD50 (83.86 mg / kg) of synthesized compound (HSV) dissolved by 0.5 ml (DMSO) intrapertoineal was given to 6 male rabbits for three weeks daily.

Group 5 (Treated 4) T4: 1/20 0f LD50 (83.86 mg / kg) of (HSV) dissolved by 0.5 ml (DMSO) intrapertoineal and 0.15ml / kg vitamin E were administered orally to 6 male rabbits for three weeks daily.

At the end of study, samples of blood were collected from the heart, 2 ml of blood was put into a tube containing the EDTA as an anticoagulant for white blood cells examinations.

White blood cells analysis

The Auto Hematology Analyzer BC5300 was used to obtain total number of white blood cells (WBC) and the differential count of white blood cells.

Statistical assay

In current experiment ,the data were expressed as mean ± SD and analyzed by using One -way analysis of Variance (ANOVA), values of $p \leq 0.05$ was considered statistically significant.

RESULTS

The results explained in Table (1) a significant decreased in total, and differential WBC count of the sulfanilamide group when compared to control group except with lymphocyte percentage. Whereas, significantly increase of total and differential WBC count of the sulfanilamide and vitamin E group as comparison to sulfanilamide except no significant increase in basophile and lymphocyte percentage.

The results in the same table showed significant increase of total, and differential WBC count of (HSV) group when comparison to sulfanilamide and vitamin E group with except no a significant increase with lymphocyte percentage. Also, there was significantly increase of WBC count, and neutrophil percentage while no significant increase in other types of WBC (basophil, eosinophil, monocyte, lymphocyte) with (HSV) and vitamin E group when comparison to (HSV) group.

Table (1): Total WBC count and differential WBC count in treated and control Groups.

parameters / groups	TWBC $X10^3$cell/ mm^3	Neutrophil %	Basophil %	Eosinophil %	Monocyte %	Lymphocyte %
Control group 0.5 ml DMSO	6.49 a ±0.44	42.24 a ±2.24	0.67 a ±0.12	1.78 a ±0.30	2.59 a ±0.25	23.49 a ±2.18
Sulfanilamide drug 195 mg/kg	4.04 e ±0.18	21.95 d ±1.52	0.08 c ±0.11	0.77 d ±0.31	0.52 d ±0.17	20.55 a ±1.20
Sulfanilamide 195mg/kgand0.15 ml/kg of vitamin E	4.76 d ±0.46	25.76 c ±1.87	0.22 c ±0.12	1.17 c ±0.14	1.33 c ±0.35	21.06 a ±1.92
HSV 83.86mg/kg	5.49 c ±0.2 0	34.79 b ±1.87	0.42 b ±0.14	1.40 b ±0.07	1.74 b ±0.16	21.65 a ±2.28
HSV 83.86mg/kg and 0.15ml/kg of vitamin E	6.01 b ±0.10	40.22 a ±1.45	0.50 b ±0.10	1.45 b ±0.24	1.96 b ±0.22	22.06 a ±1.17

Data are expressed as mean ± SD (n=6). The different letters refer to significant difference ($p \leq 0.05$).

DISCUSSION

From our results, a significant decrease in total WBC and types of WBC count with sulfanilamide group only no significant decrease in lymphocyte percentage, this finding agreed with (11), who showed the drug induced a hypo-cellular bone marrow like sulfonamide, at the same table observed significantly increased of the total white blood cell, and some WBC types only no a significant increase of the basophil and lymphocyte percentages with sulfanilamide group, and vitamin E, this may be to the antioxidant effect of vitamin E which lead to protect blood cells from damage.

This results agreed with (12) who found that vitamin E has protective effect against oxidative damage in the cell membrane from reactive oxygen species attack. These findings exhibit a significant increase of the total WBC and WBC types count except in lymphocyte percentage in treated group of (HSV) due to the ameliorative effect of HSV on WB, this results in agreement with (13) who showed that vanillin has potential antioxidant activity from ascorbic acid which is interact with radicals through a self-dimerization mechanism, the dimerization attributed to the strong reaction stoichiometry of vanillin for ROS, also observed a significant increase of the neutrophil percentage and total WBC with (HSV) and vitamin E group from (HSV) treated group caused the Vitamin E has the protective effect on the blood cells. this results which agreed with (14) demonstrated that the vitamin E treatment induced decreased the adverse effects of cadmium on the blood indices.

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