Synthesis And Characterization Of Some Bis.1,2,3,4-Tetrazoline Derivatives And Their Antioxidant Activity

Hutham Mahmood Yousif Al-Labban[1] and Ezzat Hussein Zimam[2]

University of Kufa, Faculty of Science, Department of Chemistry, Iraq.
[1]huthamm.sadiq@uokufa.edu.iq
[2]ezat ahlam@yahoo.com

ABSTRACT

In this study, we describe the synthesis of 1,2,3 and 4 tetrazoline derivatives from D-Mannitol. The initial step of the synthetic route involved obtaining azido D-Mannitol (ATN_3) as an intermediate by treating 1,2:5,6-Di-O-isopropylidene-3,4-di-p-tosyl-D-Mannitol AT with sodium azide. The intermediate (ATN_3) was subsequently reacted with Schiff base derivatives (BS_1; BS_2), leading to formation of 1,2,3 and 4-tetrazoline. The last two deprotected products (DS_1 and DS_2) were synthesized through the hydrolysis of acetal groups with dilute solution 15% AcOH. The synthesized compounds were characterized by FT-IR, $^1{}_1$H-NMR and^{13}C-NMR spectroscopy alongside Thin Layer Chromatography (TLC). The DPPH radical scavenging assay was used to assess antioxidant potential. Results indicated that derivatives DS and HS_1 are extremely potent antioxidants"Very Active," classifying them with IC_{50} of 0.42, and 4.31 µg/mL respectively, DS_1 presented a potency more than 10-fold superior to the ascorbic acid standard with fast and stable kinetics in the first 5 minutes. The results indicate that these derivatives of tetrazoline are potentially useful for further pharmacology investigations.

Keywords:1,2:5,6-Di-o-isopropylidene-3,4-di-p-tosyl-D-Mannitol,1,2,3,4-tetrazoline , azide , schiff base ,isopropyliden. DPPH.

INTRODUCTION

Methods: D-mannitol is a common naturally-occurring crystalline alditol that has been widely used both as a low-calorie sweetener and as clinical osmotic diuretic to lower in intraocular pressure [1-5]. At the same time, 1,2,3 and -4 tetrazolines are important five-membered nitrogen heterocycles that can serve as well-diversified precursors for organic synthesis through Huisgen rearrangement [6]. Such rings have attracted much attention in medicinal chemistry for their various biological activities such as antibacterial [7], anticonvulsant [8] or anti-HIV activity [9-12]. Two new tetrazoline derivatives containing D-Mannitol moieties incorporated with azo-Schiff bases of sulfadiazine were synthesized in this work. The compounds synthesized were screened for antioxidant properties by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. This is a chemical test that relies on the ability of tested compounds to quench stable DPPH free radicals, resulting in deep violet color change towards pale yellow. Spectrophotometric monitoring of the decrease in absorbance at 517 nm was used to monitor dissolution of radical concentration [13–16].

Material and Methods

Chemicals

All the chemicals were Merck-group- BDH and Fluke –German from in Sialkot used in this study.

Laboratory devices

The derivative compounds were characterized by Fourier transform infrared spectroscopy (FT.IR) (Bruker\ OPUS_7.5.1.8, Germany). .1H-NMR were recorded on Fourier transform Varian spectrometer, operating at 400 HZ.

Synthesis Methods

1-Preparation of the 1,2:5,6-Di-o-isopropylidene-3,4-bis-p-tosyl-D-Mannitol[AT] [17].

A solution of 1,2:5,6-Di-o-isopropylidene-D-Mannitol (3.3 g, 12.5 m mol) in dry pyridine (65ml) and p-toluene sulfonyl chloride (3.68 g, 23.7mmol) was added at room temperature after 6h.The mixture was poured into ice-water (400ml) and the crystalline product separated out and filtrated off, washed carefully with cold water which dried over anhydrous calcium chloride in desiccators under reduced pressure .After re-crystallization from methanol, to give [AT] compound.

Chemical Formula [AT]**:** $C_{72}H_{70}N_{20}O_{14}S_2$, Molecular Weight: 306.42, Yield= 61 %, white ppt, m.p.(109-111C^0), R_f = 0.42 (4:1 ethyl acetate: n-hexane), time of reaction= 6 hrs.

2-Preparation of the 1,2:5,6-di-o-isopropylidene-3,4-diazido-D-Mannitol [ATN_3] [18].

To the solution of 1,2,5,6-Di-o-isopropylidene-3,4-bis-p-tolyl sulfonyl-d-mannitol [AT] (1.2g ,2.5mmole) in N,N-Dimethyl formamide (2ml), water (1.5ml) ,sodium azide (0.325, 5mmol), and urea (0.8 g,10.5mmole),were added. The mixture was heated for (27h) at 110C^0under nitrogen atmosphere. The cooled solution was poured into ice water (600ml),and then was extracted with chloroform(3x10ml).Organic Layers were combined and washed with aqueous sodium bicarbonate (10ml), and dried over anhydrous magnesium sulfate .The solvent was evaporated under reduced pressure to give[ATN_3]compound .

Chemical Formula [ATN_3]: $C_{10}H_{20}N_6O_4$, Molecular Weight: 288.31, Yield = 73 %, gray gummy, R_f= 0.47 (1:4 ethyl acetate: n-hexane), time of reaction= 27 hrs.

3-General procedure of synthesis of 4-amino-N- (pyrimidin-2-yl)-3-(p- tolyldiazenyl) benzenesulfonamide [B] [19].

A solution of p-toluidine mixture (1.06 g, 0.01 mol) with hydrochloric acid concentrated (4 mL) and distilled water (20 mL); cooled to 0°C using ice-water bath apparatus was prepared first in a beaker; Then, a solution of sodium nitrite (0.68 g, 0.01 mol) in distilled water (6 mL) was added dropwise with stirring to yield the corresponding diazonium chloride .
Sulfadiazine (2.5 g, 0.01 mol) were dissolved in sodium hydroxide (2 g) and distilled water (20 mL). The production reaction is kept at 0 °C in the ice-water bath. The cold diazonium chloride solution freshly produced (15 mL) was added, during strong stirring after each addition in the sulfadizine solution to couple efficiently. The reaction mixture was stirred at 0 °C for an additional 15 min after complete addition.
The brown precipitate that formed was filtered, washed with water to give compound [B] as a solid which was dry in air and then purified through recrystallization from ethanol into compound [B].

Chemical Formula: $C_{17}H_{16}N_6O_2S$,Molecular Weight:368g\mol, yield 78% , m.p = (258) C^0, Brawn ppt., and Rf =(0.74).

4- Synthesis of Schiff bases derivatives [BS_1-BS_2] [20].

The azo compound [B] (0.01 mol) was added to a solution of vanillin or p-hydroxybenzaldehyde (0.01 mol) in absolute ethanol (40 mL). Two drops of glacial acetic acid were also added to the reaction mixture. The mixture was refluxed for 23–25 h.
The formed precipitates were collected by filtration, dried, and recrystallized from ethanol. The progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate:cyclohexane (4:1) as the eluent system, confirming the formation of compounds [BS_1–BS_2].

Chemical Formula [BS_1]: $C_{25}H_{22}N_6O_4S$;Molecular Weight:502,Yield=67%,brawn ppt.,m.p. = (274-276)℃, R_f =0.45 (4:1 ethyl acetate: n-hexane), time of reaction = 23 hr.

Chemical Formula [BS_2]: $C_{24}H_{20}N_6O_3S$; Molecular Weight: 472.Yield= 71 % ,orange ppt. ,m.p. =250℃ dec, R_f =0.34 (4:1 ethyl acetate:n-hexane),time of reaction = 25 hr.

5- Synthesis of 1,2,3-tetrazoline derivatives[HS_1 and HS_2] [21].

To a solution of azido sugar (ATN_3) (0.01 mol) in DMSO: D.W.(1:1), the chalcone derivative [Bch_1-Bch_5] and schiff base derivative [BS_1-BS_5] (0.01mol), cupric sulfate pentahydrate (1.78 mol, 0.444 g) and sodium ascorbate (1.78 mol, 0.35 g) were added, and the mixture was refluxed. The reaction controlled by TLC (ethyl acetate: cyclohexane 1:4). The solvent was reduced by a rotary evaporator, extracted by $CHCl_3$, the organic layer dried by anhydrous sodium sulfate (Na_2SO_4), filtered, and the solvent was removed in vacuum. The crude material was purified by flash column chromatography (ethyl acetate: hexane 1:1).

Chemical Formula [HS_1]: $C_{74}H_{76}N_{18}O_{14}S_2$, Molecular Weight:1505.65, Yield= 62 %, brawn ppt, m.p. = (105 dec)℃,R_f= 0.35 (4:1 ethyl acetate: n-hexane), time of reaction= 105 hrs.
Chemical Formula [HS_2]: $C_{72}H_{72}N_{18}O_{12}S_2$; Molecular Weight: 1445.60, Yield=70 %, brawn ppt, m.p. = (108dec)℃, R_f=0.32 (4:1 ethyl acetate: n-hexane), time of reaction= 117 hrs.

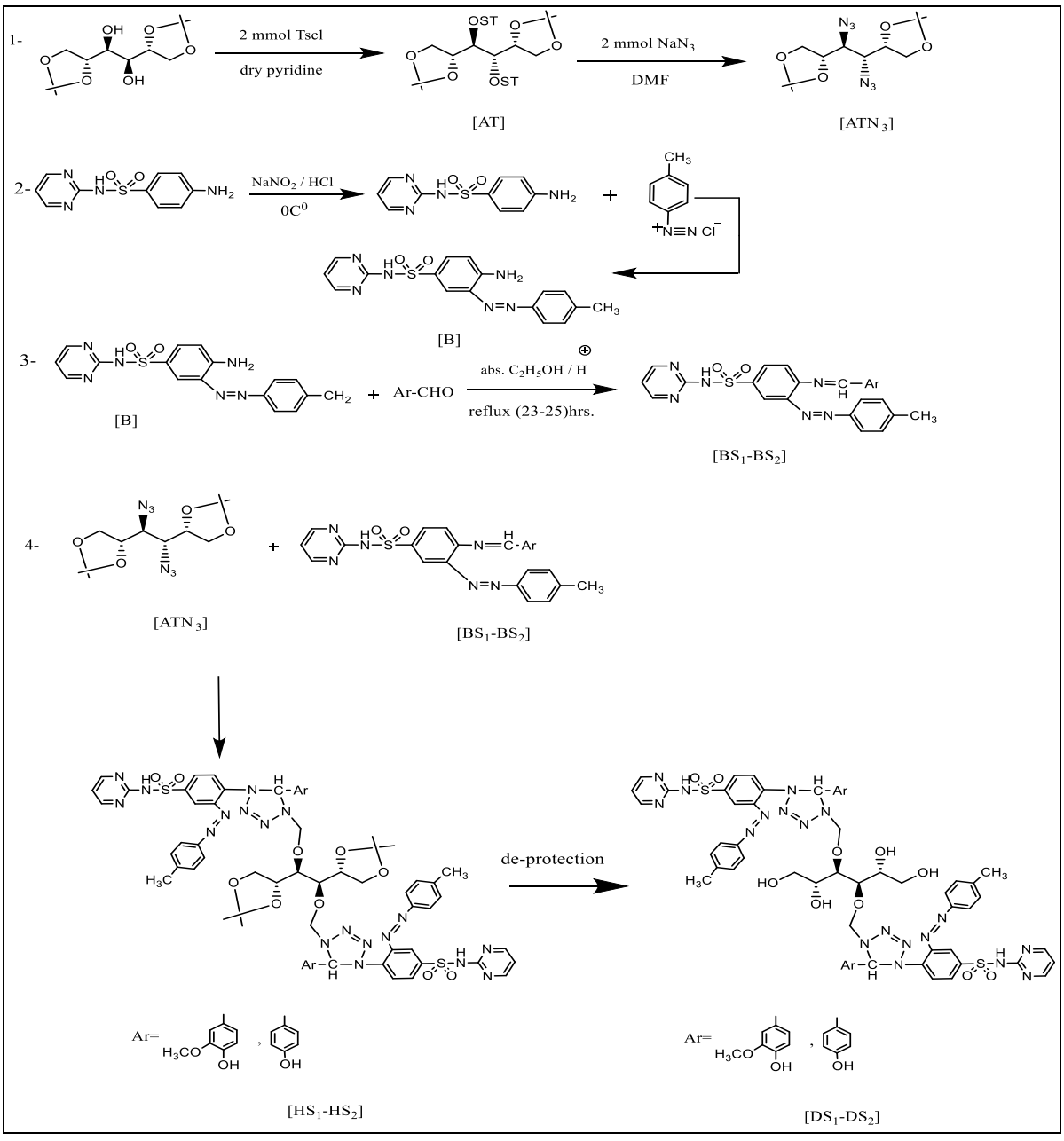
6-General procedure for de-isopropilidine of compounds [DS_1 and DS_2] [18]

(0.01 mol) isopropidine-sugar [HS_1 and HS_2] dissolved in (30 mL) dilute acetic acid (15%),stirred until disappearance of starting material from TLC.added cold D.W., extracted, organic layer dried over Na_2SO_4, and reduced in vacuum to give[DS_1-DS_5].

Chemical Formula[DS_1]:$C_{70}H_{68}N_{18}O_{14}S_2$, Molecular Weight: 1449.55, Yield= 60 %, orange ppt, m.p. = (118 dec),R_f= 0.41 (4:1 toluene: ethanol), time of reaction= 125 hrs.

Chemical Formula[DS_2]:$C_{68}H_{64}N_{18}O_{12}S_2$;Molecular Weight:1389.49,Yield=72 %, orange ppt, m.p. = (112 dec),R_f = 0.32 (4:1 toluene: ethanol), time of reaction= 45 hrs.

Scheme of reactions:



Scheme 1: Synthesis of D-mannitol bistetrazoline

Antioxidant assay

Preparation of solutions [22]

DPPH radical solution (3mg/100mL) was prepared by dissolving DPPH in methanol and diluting to 1000 mL in a volumetric flask. Stock solutions of the tested compounds(DS_1- HS_1) 75 µg/mL were prepared similarly. Sub-stock solutions (50 µg/mL and 25 µg/mL) were then prepared by diluting the corresponding stock solutions to 10 mL with methanol.

Determination of inhibition and IC_{50}[23]

Equal volumes of the sample solutions (50 µg/mL 25 µg/mL)and DPPH solution were mixed. After incubation for 5, 10, and 15 min, the absorbance was measured at 517 nm. $\%\ \boldsymbol{inhibition\ of\ activity\ of\ DPPH} = \frac{A-B}{A} * X\ 100$……. equation (1)

Where A is the control (DPPH and methanol) absorbed density and B is the sample absorbed density.

The concentration required to inhibit 50% of DPPH radicals (IC_{50}) was determined from the inhibition curve.

RESULTS AND DISCUSSION

Compound [AT]

TheF.T.I.R spectrum showed disappearance of the OH bond absorption at 3406 $cm^{-1}$and appearance **:** (υCH $_{alph.}$) 2989, 2922,(υSO_2 **asy** &sy) 1372,1160,(υC-O-C)1058.seefigure(1).

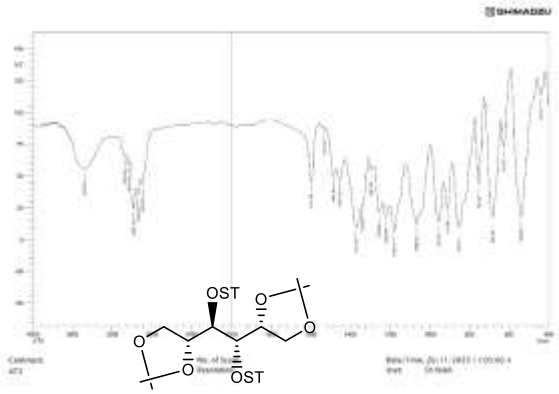


Figure (1): F.T.I.R spectrum of compound (AT)

Compound[ATN_3] showed appearance (υCH $_{aliph.}$) 2933,2980(SO_2 **asy** &sy)1373 ,1160, (υC-O-C) 1093, (υN_3) 2115 ,1068, (υC-N) 1263 .see figure (2).

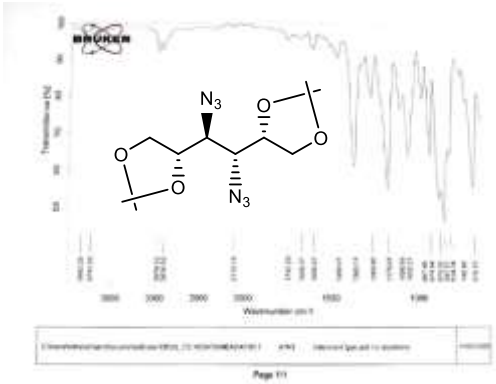
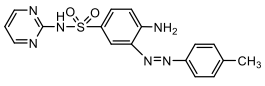


Figure (2): F.T.I.R spectrum of compound (ATN_3)

Compounds[B]

The FT-IR spectrum showed the appearance (N=N) 1435 cm^{-1},(NH_2 amine sulfonamide) 3348, (υ N-H $_{pyrimidine}$)3252, (υ $CH_{Ar.}$)3029 , (υ C=N) 1647, (υ C=C $_{aromatic}$) 1489(υC-N)1255, (υ N=N) 1435,(υC-S) 801, (υSO_2asy&sy) 1317,1146, (υ CH_3 methyl $_{group}$) 2933,2863.see figure (3).



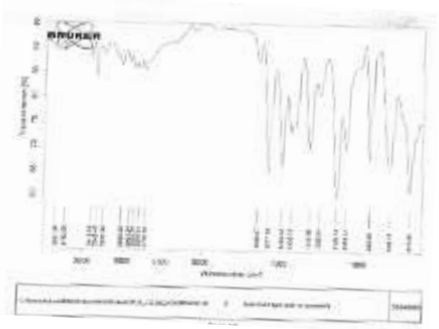


Figure (3) : F.T.I.R spectrum of compound [B].

Compounds [$BS_1$and BS_2]

FT-IR spectra of compounds [BS_1–BS_2] displayed a typical absorption band between 1580 and 1655 cm^{-1}, which was assigned to stretching vibration (C═N) in imine. The other absorption bands appeared in the range between 3420–3252 cm^{-1} was attributed to stretching vibration of secondary sulfonamide (N-H) group. The relevant spectral changes provide compelling support for the successful preparation of these Schiff base compounds. The main functional group absorptions are media-specific and summarized in Table (1). FT-IR spectra (KBr,cm^{-1}) of [BS_1–BS_2] Compounds are showed in Figures(4–5).

Table (1): FT-IR bands of compounds (BS_1-BS_2)

Comp.	υ(CH)Ar.	υ(CH) aliph.	υ(NH)	υ($SO_{2asy\&sy}$)	υ(C=N) imin	υ(N=N)	υ(C-S)	υ(C-N)	Others
BS_1	3034	2866 2934	3252	1319-1146	1580	1437	676	1261	(υ OH) 3355 υ(Ar-OCH_3) :2866
BS_2	3033	2921 2857	3420	1320,1146	1655	1492	677	1277	(υ OH) 3354

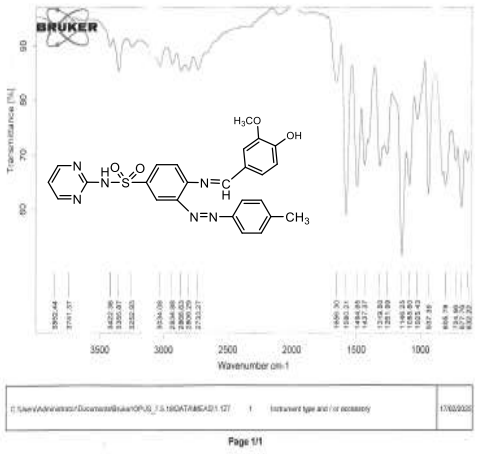


Figure (4): F.T.I.R spectrum of compound [BS_1]

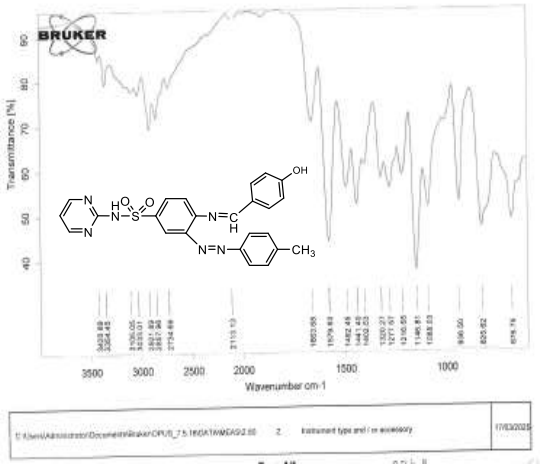


Figure (5): F.T.I.R spectrum of compound [BS_2]

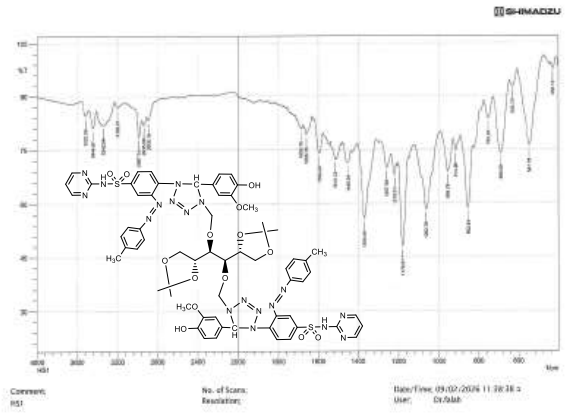
Compounds [HS_1 and HS_2].

New series of 1,2,3,4-tetrazoline derivatives [HS_1 and HS_2] via the reaction between azide compound (ATN_3) with substituted Schiff bases (BS_1 & BS_2) in presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate using a catalyst system to give 1,2,3,4-tetrazoline derivatives.

The FTIR spectrum shows Compounds (HS_1andHS_2) identified by the disappearance of peak at 2108cm^{-1} for the azide group for starting materials (ATN_3) and the appearance peaks for new tetrazoline derivatives as shown in table (2) and figures (6-7)

Table (2): Infrared spectra data for compounds [HS_1 and HS_2]

Comp.No	NH	ν(C-H) Aliph	ν(C=C)	ν(N=N)	ν(C-N)	Others
HS_1	3252	2934-2866	1593	1437	1261	**υ**(OH)3355, υ(ArOCH3) Str. : 2866
HS_2	3420	2981-2933	1593	1492	1263	**υ**(OH)3288



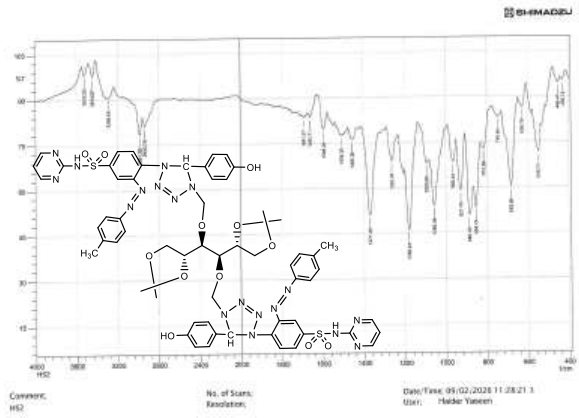


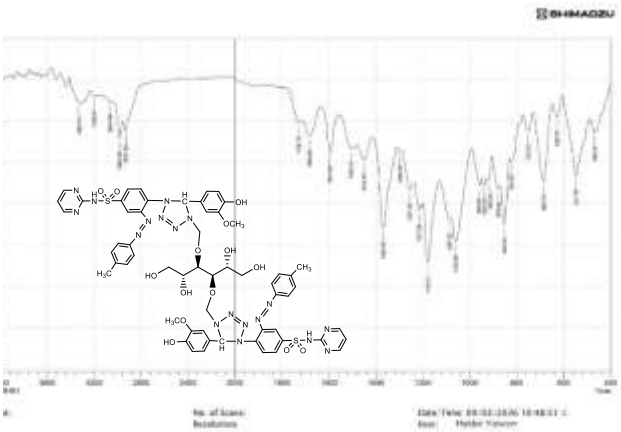
Figure (6): F.T.I.R spectrum of compound (HS_1) **Figure (7):** F.T.I.R spectrum of compound (HS_2)

Compounds [DS_1 and DS_2].

General de-protection of compounds [DS_1 and DS_2]by using dilute acetic acid (15%).The FTIR spectroscopy shows the appearance of hydroxyl peak in (3327-3300) cm^{-1}.The other bands are shown in table (3) and figures (8-9.

Table (3): FTIR spectrum data of compounds [DS_1 and DS_2]

Comp.No	υ(OH) υ(NH)	υ(CH) aliph.	υ(CH) Ar.	υ(C=C)	υ(N=N)	υSO_2 asy&sy.	ν(C-N)	Others
DS_1	3327	2983 2931	3064	1597	1508	1369 1178	1257	υ(Ar-OCH_3) Str. : 2931
DS_2	3300	2981 2931	3062	1593	1489	1371 1180	1261	-



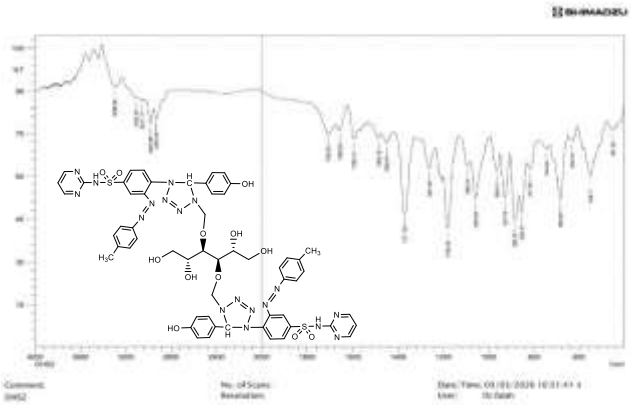


Figure (8): F.T.I.R. spectrum of compound (DS_1) **Figure (9):** F.T.I.R spectrum of compound (DS_2)

^{1}HNMR (400 MHz, DMSO-d6)[DS_1]δ(ppm) :11.57(NH-SO_2), 8.56 (OH,CH- tetrazoline rings), 7.87 -7.90 (Ar-H), 3.17 (sugar backbone protons CH, CH_2)4.57 (OH sugar backbone protons), 2.30 (CH_3). See figure(10).

^{1}HNMR (400 MHz, DMSO-d6)[DS_2]δ(ppm) :11.35(NH-SO_2), 9.17 (OH,CH-tetrazoline rings), 6.5-7.8 (Ar-H), 3.3 (sugar backbone protons CH, CH_2),5.2(OH sugar backbone protons), 2.58 ($2CH_3$). See figure (11).

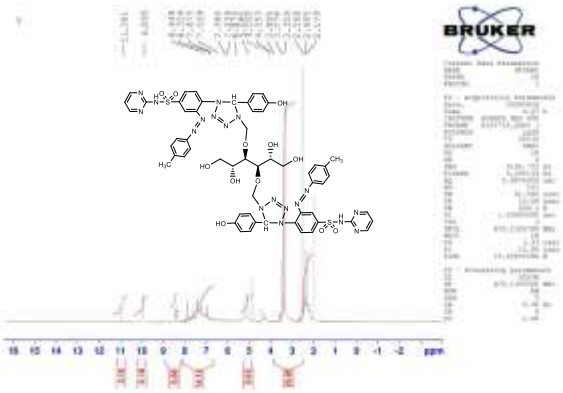


Figure (10): ^{1}HNMR spectrum of compound (DS_1)

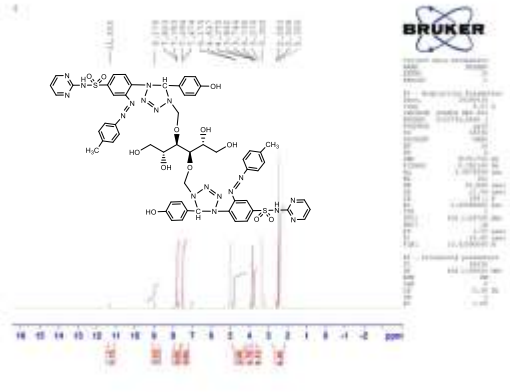


Figure (11): ^{1}HNMR spectrum of compound (DS_2)

^{13}C-NMR (400 MHz, DMSO-d6) δ(ppm) [DS_1] :169.98, (C=N), 116.16- 149.65 (Ar-C), 85.32 (N-CH-N,tetrazoline),65.64-78.5(sugar carbons),40.19 (N(CH_3 overlapping with solvent),21.61 (Ar-CH_3) , See figure(12).

^{13}C-NMR (400 MHz, DMSO-d6) δ(ppm) [DS_2] :169.98, (C=N), 116.16- 149.65 (Ar-C), 85.32 (N-CH-N,tetrazoline),65.64-78.5(sugar carbons),40.19 (N(CH_3 overlapping with solvent),21.61 (Ar-CH_3) ,See figure(13).

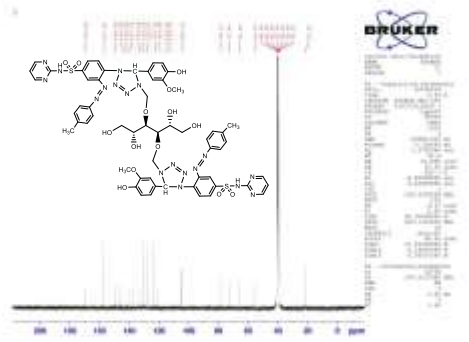


Figure (12): ^{13}C-NMR spectrum of compound (DS_1)

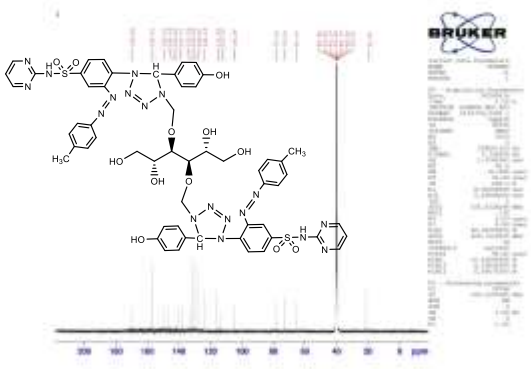


Figure (13): ^{13}C-NMR spectrum of compound (DS_2)

Anti-Oxidant activates

The DPPH Radical Scavenging was used to evaluate the antioxidant capacity of sulfa drug ($DS_1$and HS_1) derivatives (50 µg/mL) in methanol Absorbance was recorded at λ_{max}=517 nm. at 5, 10, and 15 determined by comparing the absorbance of the samples against control (1.119).

Table (4) show Absorbance and Inhibition % of ($DS_1$and HS_1) at 5, 10, and 15 min.

Table (4): Absorbance and Inhibition % of ($DS_1$and HS_1) derivatives at 5, 10, and 15 min

Control Absorbance =1.119 λ = 517 nm							
Compounds		After 5 min		After 10 min		After 15 min	
		Sample	% Inhibition	Sample Abs.	% Inhibition	Sample Abs.	% Inhibition
DS_1	50µg/mL	0.338	69.7944	0.334	70.1514	0.333	70.2412
	25µg/mL	0.349	68.8114	0.347	68.9901	0.348	68.9008

HS_1	**50µg/mL**	0.258	76.9437	0.216	76.6756	0.257	77.0330
	25µg/Ml	0.249	77.7479	0.247	77.9267	0.246	78.0160

The antioxidant evaluation of the synthesized tetrazoline derivatives showed that HS_1 (at 25µg/mL) exhibited the highest radical scavenging activity, achieving a peak inhibition of 78.01% within 15 minutes. Notably, HS_1 displayed rapid kinetics, reaching near-maximal inhibition in the first 5 minutes. For instance, DS1 (at 50 25µg/mL), exercised a lower inhibiting pattern of only around70.24 %. All of these results indicate that the hydrogen-donating ability of DS_1 analogue was greater as conferred by structural modifications in HS1 derivative. Figure (14) demonstrates the standard calibration curve of DS_1-HS_1 derivatives.

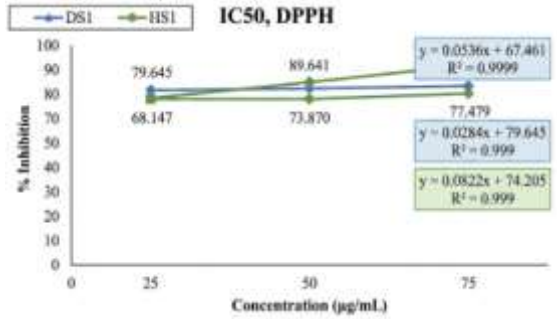


Figure 14: The Standard Calibration Curve of ($DS_1$and Hs_1) derivatives

2- Inhibitory Concentration Value 50% (IC_{50})

Notably, HS_1 exhibited exceptional activity with an IC_{50} of 0.42 µg/mL, outperforming both DS_1 (4.31 µg/mL) and the standard Ascorbic acid (4.21 µg/mL). Based on standardized classification, both derivatives were classified as "Very Active" (IC_{50} < 10 µg/mL). The extremely low IC_{50} of HS_1 is indicative the ability to optimise its chemical structure as it appears highly effective in effectively deactivating DPPH free radicals, considerably more than with reference commercial agents. Table (5): Linear Regression and IC_{50} Values of (DS_1 and HS_1) derivatives and Ascorbic acid.

Table (5): Linear Regression and IC_{50} Values of (DS_1 and HS_1) derivatives and Ascorbic acid.

Comp.	Linear Equation	Regression	IC_{50} (µg/mL)
Ds_1	y = 0.0536x + 67.461	R^2 = 0.9999	4.31
HS_1	y = 0.0108x + 77.479	R^2 = 0.9999	0.42
Ascorbic acid	y = 0.1654x + 48.122	R^2 = 0.9885	4.215

REFERENCE

[1] Naderi, A., Gobbi, N., Ali, A., Berjisian, E., Hamidvand, A., Forbes, S. C., ... & Saunders, B. (2023). Carbohydrates and endurance exercise: A narrative review of a food first approach. Nutrients, 15(6), 1367.

[2] Margolis, L. M., & Pasiakos, S. M. (2023). Low carbohydrate availability impairs hypertrophy and anaerobic performance. Current Opinion in Clinical Nutrition & Metabolic Care, 26(4), 347-352.

[3] Chen, M., Zhang, W., Wu, H., Guang, C., & Mu, W. (2020). Mannitol: physiological functionalities, determination methods, biotechnological production, and applications. Applied Microbiology and Biotechnology, 104, 6941-6951.

[4] Burness, C. B., & Keating, G. M. (2012). Mannitol dry powder for inhalation: in patients with cystic fibrosis. Drugs, 72, 1411-1421.

[5] Bazyka, D. A., & Lytvynenko, O. O. (2022). CLASSIFICATION OF MEDICAL EQUIPMENT FOR ANTI-RADIATION PROTECTION. Problemy Radiatsiinoi Medytsyny ta Radiobiolohii, 27, 84-106.
[6] P. Gupta, A., D. Sharma,"Pharmacological Significance of Triazoles and Tetrazoles in Neurodegenerative Disease: An Overview",N-Heterocycles: Synthesis and Biological Evaluation,vol. 2, no. 7, pp. 355-393, 2022.
[7] Alsaeedy, M., Arwa, A. A., Alrabie, A., Al-Qadsy, I., Khamees, H. A., Alaizeri, Z. A. M., ... & Farooqui, M. (2023). Novel hybrid triazoline-triazole glycosides: synthesis, characterization, antimicrobial activity study via in vitro, and in silico means. Carbohydrate Research, 532, 108877.
[8] Chaidam, S., Saehlim, N., Athipornchai, A., Sirion, U., & Saeeng, R. (2021). Synthesis and biological evaluation of 1, 6-bis-triazole-2, 3, 4-tri-O-benzyl-α-d-glucopyranosides as a novel α-glucosidase inhibitor in the treatment of Type 2 diabetes. Bioorganic & Medicinal Chemistry Letters, 50, 128331.
[9] Mishra, V. K., Khanna, A., Tiwari, G., Tyagi, R., & Sagar, R. (2024). Recent developments on the synthesis of biologically active glycohybrids. Bioorganic Chemistry, 145, 107172.
[10] Mishra, V. K., Khanna, A., Tiwari, G., Tyagi, R., & Sagar, R. (2024). Recent developments on the synthesis of biologically active glycohybrids. Bioorganic Chemistry, 145, 107172.
[11] Mishra, D. R., Panda, B. S., Nayak, S., Rauta, N. K., Mohapatra, S., Sahoo, C. R., & Padhy, R. N. (2022). One-pot multicomponent synthesis of 4-((2H-chromen-3-yl)/(2-phenyl-2H-chromen-3-yl) methylene)-3-methylisoxazol-5 (4H)-ones and evaluation of their antibacterial activity. Tetrahedron, 124, 133015.
[12] Panda, B. S., Ahemad, M. A., Mohapatra, S., Naik, E., Nayak, S., Mohapatra, S., ... & Sahoo, M. K. (2024). Microwave-assisted click synthesis, characterisation, and In silico studies of novel 2H-chromene-1, 2, 3-triazolyl glycoconjugates as potent anticancer and antibacterial agents. Journal of Molecular Structure, 1318, 139323.
[13] Nakanishi, I., Shoji, Y., Ohkubo, K., Ito, H., & Fukuzumi, S. (2023). Water-induced regeneration of a 2, 2-diphenyl-1-picrylhydrazyl radical after its scandium ion-promoted electron-transfer disproportionation in an aprotic medium. Molecules, 28(13), 5002.
[14] Sahib, H. B., Aldhalmi, A. K., Hassan, O. M., Mahmood, A. A. R., Tahtamouni, L. H., & Ali, Z. K. (2025). Anti-Angiogenic and Anti-Proliferative Activities of 5-Bromo-N-(2, 5-Dioxopyrrolidin-1-Yl)-1H-Indole-2-Carboxamide. Asian Pacific Journal of Cancer Prevention: APJCP, 26(4), 1219.
[15] Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J., & Gotoh, H. (2024). DPPH measurements and structure—Activity relationship studies on the antioxidant capacity of phenols. Antioxidants, 13(3), 309.
[16] Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. Processes, 11(8), 2248.
[17] Juber, I. K. (2017). Synthesis and Characterization of 1, 2, 3-Triazole Derivatives from D-Mannitol. Tikrit Journal of Pure Science, 22(9), 58-68.
[18] Rawa H, yaqoob ,T.A.Fahad , J of world of pharmaceutical Research , 5,01,35-43 ,(2015).
[19] Josephy, P. D., & Allen-Vercoe, E. (2023). Reductive metabolism of azo dyes and drugs: Toxicological implications. Food and Chemical Toxicology, 178, 113932.
[20] J.R .Ancona ,Natiana Noriega ,Juan Gamus , J of Spectrochimica Acta part A : Molecular and Biomolecular Spectroscopy , Vol 137,16-22 ,(2015).
[21] Alonso, F., Moglie, Y., Radivoy, G., & Yus, M. (2011). Multicomponent click synthesis of 1, 2, 3-triazoles from epoxides in water catalyzed by copper nanoparticles on activated carbon. The Journal of Organic Chemistry, 76(20), 8394-8405.
[22] Mishra, K. B. (2025). Ene–azide chemistry in the synthesis of 1, 2, 3-triazoline/triazole and the corresponding mechanistic aspects. Organic & Biomolecular Chemistry, 23(23), 54
[23] Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules, 27(4), 1326.