

Synthesis, Characterization, And Antioxidant Activity Of 1,2,3-Triazole Derivatives From 5,6 -Isopropylidene-L-Ascorbic Acid

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ABSTRACT

The need to develop powerful antioxidant agents capable of combating resistant infections without compromising human health has motivated significant research for novel antioxidant compounds[1] .

In this study two of Sulfadiazine Azo azides derivatives (2,4) are reacted with compound (A) 5,6- isopropylidene-2,3-di-O-propargyl-L-ascorbic acid to prepare a new 1,2,3-triazole derivatives (A2 and A4) derivatives are synthesized by Copper (I) catalyzed azide-alkyne cycloaddition reaction (Click Chemistry)². Following the synthesis, the de-protected analogs (A22 and A44) were obtained by removing the acetal groups using Iodine in methanol. All synthesized compounds were structurally distinguished using FT-IR, ¹H-NMR, ¹³C-NMR, and TLC techniques.

The synthesized compounds A4,A44 exhibited outstanding antioxidant efficiency with IC₅₀ values below 25 $\mu\text{g/mL}$, significantly outperforming the standard Ascorbic acid and highlighting their promising potential as potent biomaterials.

KEYWORDS:1,2,3-triazoles, 5,6-isopropylidene-L- ascorbic acid, azide, Sulfadiazine derivatives, Click chemistry, Azo compound.

1.INTRODUCTION

Reactive oxygen , Nitrogen species ROS/RNS and free radicals which are created in vivo continuously being formed in aerobic organisms have the main role of the oxidative damage to protein, lipid membranes and DNA in cells and tissues[2] and are therefore involved in the development or expansion of several kind of diseases[3,4]Under standard conditions they are removed by enzymatic and non enzymatic pathway antioxidant immune systems[5,6].

L-Ascorbic acid is one of the naturally eventuated a vital antioxidant and free radical scavengers that shows preserve action cellular constituent against oxidative damage mechanism by free radicals and active oxygen and Nitrogen species[6-8].

The high radical scavengers activity of L-Ascorbic acid is caused by the presence of the conjugated system in (1-oxo-ene-2,3-diol) ,this important properties is widely applied in the pharmaceutical and industrial application[9-11], Vitamin C (L-Ascorbic acid) also plays an important role in the preventing of a large number of chronic diseases such as cancer, cerebral apoplexy, diabetes mellitus, MI and AIDS[12-18].

Triazoles are an important heterocyclic compound . They are a five membered ring that containing three nitrogen atom they are found in two isomeric forms i.e 1,2,4-triazole and 1,2,3-triazole they are used as creation of new therapeutic due to a biomedical application, such as antibacterial ,antiviral, antifungal ,antimalarial, antiallergic ,antitubercular ,CNS depressant antihypertensive, analgesic, antiproliferative agents[1,19-23].

antimicrobial resistance is the main problem at present time that,our medical field is suffering from it,for that the expansion research to produce new antimicrobial compounds that may be exhibiting effectiveness against medicine[25-27].

,also triazoles have an antioxidant properties evaluated by DPPH assay, an easy rapid way that evaluate the antiradical activities of antioxidants by a spectral method .

In this study, a novel triazole rings were synthesized by reacting isopropylidene L-Ascorbic acid with Azo sulfadiazine derivatives based azides to investigate their potential pharmaceutical enhancements.

Scheme :

Chemical Formula[3]: $C_{16}H_{13}N_6O_2SBr$, Molecular Weight :433.28g \mol, yield 85% , m.p. 250 °C, Brown ppt., and $R_f=0.77$ (ethyl acetate:cyclohexane,1:4).

2.3.3-General procedure of synthesis of 4-azido-3-((4-bromophenyl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide [4] [29]

(16.9 mmol,7.322g) of compound [3] is dissolved in a mixture of distilled water and Hydrochloric acid in a percentage of (1:2.5) mL , the mixture was cooled to 0°C by a salt-ice bath

then a cold aqueous solution of sodium nitrite (16.9mmol,1.171g) is added to sulfadiazine solution drop by drop where after several additions the color of the solution turned to a greenish brown color, after complete the addition the mixture is left on the stirring for 45 min, then an aqueous solution of sodium azide was prepared (2eq, 2.207g) ,then added in a small portion, a bubbles were observed during the addition. After the complete of addition , the solution is left for stirring for (2 hrs).The brown formed precipitate is filtered then washed several times with distilled water.

Chemical Formula [4]: $C_{16}H_{12}N_9O_2SBr$;Molecular Weight: 474.30 g/mol Yield=66%,Brown ppt.,m.p. = (164) °C , R_f = 0.22(ethyl acetate:Cyclohexane, 4:1).

2.3.4-General procedure of synthesis of N-(2-((4-bromo phenyl) diazenyl)-4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)-2-chloroacetamide [5][30].

Chloroacetylchloride (15mmol,1.7mL) was added dropwise to a stirrer mixture of Compound [3] (10 mmol, 4.33g) and K_2CO_3 (15 mmol, 2g) in DMSO (100mL) at 0°C after complete addition the reaction stirred at room temperature for 42 hrs, monitored by TLC using (ethyl acetate: Cyclohexane 4:1).After completing, the reaction added to cold D.W, extracted, dried by anhydrous sodium sulfate (Na_2SO_4),filtered,and concentrated by rotary evaporator.

ChemicalFormula[5]: $C_{18}H_{14}ClN_6O_3SBr$;Molecular weight509.76 g/mol, Yield=69%, brown ppt. ,m.p.=(164)°C. R_f = 0.4 (ethyl acetate: Cyclohexane 4:1).

2.3.5-General procedure of synthesis of 2-azido-N-(2-((4-bromo phenyl)diazenyl)-4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)acetamide [6][31].

[5] (22.11 mmol, 11.27g) and sodium azide (18 mmol,1.17g) in DMSO (15 mL)stirred at room temperature for 72hr ,monitored by TLC using (ethyl acetate :n-hexane 1:1).After completing the reaction, added to cold D.W., extracted, dried by anhydrous sodium sulfate (Na_2SO_4),filtered and concentrated by rotary evaporator.

Chemical Formula[6]: $C_{18}H_{14}N_9O_3SBr$;Molecular weight:451.47g/mol ,Yield=70%, brown ppt.,m.p. (125) °C. R_f =0.45 (1:1 ethyl acetate:n-hexane).

2.3.6-General procedure for Synthesis of 1,2,3-triazole derivatives [A4,A6][23].

A propargylic ether [A](0.264 g, 1.0 mmol) in DMSO (10 mL) in a round bottomed flask , a suspension of sodium ascorbate (0.0396 g, 0.2 mmol) and $CuSO_4 \cdot 5H_2O$ (0.025 g, 0.01 mmol) in DMSO (20mL) was added to the solution in the round , the mixture was stirred for 10 min. Next,To solution of azide derivatives (4 and 6)(0.01mol) in DMSO (30 mL) was added, and the mixture heated at 60 °C with stirring for 38-75 hrs. The reaction was controlled by TLC (1:1 ethanol: n-hexane).The mixture was diluted with H_2O (30 mL), extracted with EtOAc (3 × 30 mL), the combined organic layers washed with saturated NaCl (2 × 20 mL),dried over Na_2SO_4 and evaporated under reduced pressure. The residue was flash chromatographed (silica gel, n-hexane / EtOAc; 2:1 → 1:2,) to yield the corresponding bis-1,2,3-triazole.

Chemical Formula[A4]: $C_{47}H_{38}N_{16}O_{10}Br_2S_2$,MolecularWeight:1210.85 ,Yield=64 % , gummy Dark Brown ppt. , , R_f =0.61 (1:1 ethanol: n-hexane), time of reaction= 40hrs.

Chemical Formula [A6]: $C_{51}H_{44}N_{18}O_{12}S_2Br_2$, Molecular Weight: 1324.96 g/mol, Yield=60%, Brown ppt. ,m.p.= (121)°C, R_f =0.14 (4:1 ethanol: n-hexane), time of reaction= 75 hrs

2.3.7-General procedure for de-isopropylidene of compounds [A44 and A66][32].

(0.01 mol)5,6-O- isopropylidene bis-2,3-O-triazole –L-Ascorbic acid derivatives [A4 and A6] dissolved in a little amount of Chloroform then reflux the mixture with a solution of Iodine in methanol (1%) W/V, stirred until disappearance of starting material from TLC after (12-14 hrs.), added Sodium Thiosulfate to reduce unreacted Iodine ,vapour the solvent ,extracted with ethyl acetate, organic layer dried over Na_2SO_4 ,and reduced in vacuum.

Chemical Formula[A44]: $C_{44}H_{34}N_{16}O_{10}S_2Br_2$, Molecular Weight: 1170.85 ,Yield=70 % , brown gummy ppt. , , time of reaction= 12 hrs.

Chemical Formula[A66]: $C_{48}H_{40}N_{18}O_{12}S_2Br_2$, MolecularWeight: 1284.96 ,Yield=72%,brown gummy ppt., time of reaction=14 hrs.

2.3.8-Antioxidant assay:

2.3.8.1-Preparation of DPPH (3mg/100mL) solution[33]:

30mg of DPPH dissolve in (1000 mL) beaker with 200mL of methanol. The volume was completed to the mark (1000 mL) with same solvent after transferred the solution to (1000 mL) volumetric flask.

2.3.8.2- Preparation stock solution (75 µg/mL) of Sample solution:

75 µg/mL of compounds (A4 and A44) prepared by dissolve 75 mg in a (1000 mL) beaker with 200mL of methanol. The volume was completed to the mark (1000 mL) with same solvent after transferred the solution to (1000 mL) volumetric flask.

2.3.8.3- Preparation (50 µg/mL) of Sample solution:

Transferred a (6.667 mL) stock solution to a (10 mL) volumetric flask and complete the volume by methanol to the mark.

2.3.8.4- Preparation (25 µg/mL) of Sample solution:

Transferred a (3.333 mL) stock solution to a (10 mL) volumetric flask and complete the volume by methanol to the mark.

2.3.8.5 -Determination of inhibition:

Mixing equal amount of sample solution (50 µg/mL) and DPPH solution tested intervals of 5, 10, and 15 min, the absorbance measured at $\lambda_{\max}=517\text{nm}$. The % inhibition was calculated by apply equation (1).

$$\% \text{ inhibition of activity of DPPH} = \frac{A-B}{A} * 100 \dots \text{equation (1)}$$

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

2.3.8.6 -Inhibitory Concentration Value 50% (IC₅₀)[33]:

After mixing equal amount of sample solution 25 µg/mL and DPPH solution, the absorbance measured at $\lambda_{\max}=517 \text{ nm}$ and repeat same procedure with 50 and 75 µg/mL. The % inhibition was calculated by apply equation (2). The sample concentration draw in x-axis and the percentage of inhibition on y-axis. From the equation $y=bx-a$ the value of IC₅₀ calculated by using equation (2).

$$IC_{50} = \frac{50-a}{b} \dots \text{Equation (2)}$$

Where **a** = Intercept

b = Slope

3.RESULTS AND DISCUSSION

3.1 Compound [A]: In the first approach the conversion of 5,6-O-isopropylidene –L-ascorbic acid to [A]compound was investigated by FTIR, The FTIR spectrum figure (1) show the appearance of new alkyne carbon-carbon bands at $(3286) \text{ cm}^{-1}$ for terminal alkyne-carbon-hydrogen ($\equiv\text{CH}$), a new band at (2100 cm^{-1}) ($\text{C}\equiv\text{C}$), a band at $(2987, 2850) \text{ cm}^{-1}$ for $\nu(\text{CH})_{\text{aliph.}}$, and $(1058) \text{ cm}^{-1}$.

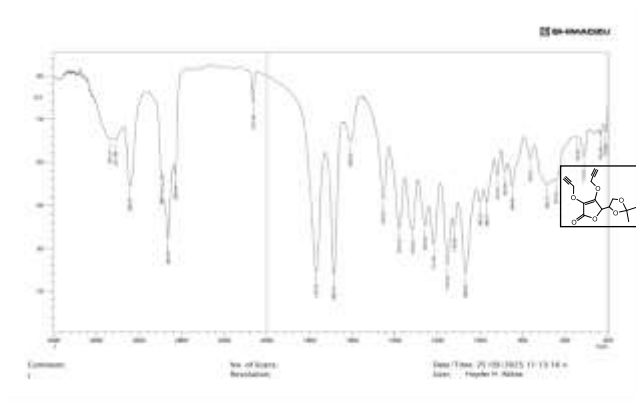


Figure (1): FTIR spectrum of compound [A]

3.2 Compound [3]: The Key intermediate compound [3] was made first by converted sulfadiazine to the sulfadiazine diazonium chloride by reaction with concentration hydrochloric acid and sodium nitrite. Diazonium salt was directly introduced in a coupling reaction with P-Bromo aniline to produce Azo sulfadiazine[3]. The FTIR spectrum figure (2) show the appearance of two absorption band, of the asymmetric and symmetric stretching vibration of (NH_2) group of sulfadiazine and spectra of these compounds showed the disappearance of two absorption band, of the asymmetric and symmetric stretching vibration of (NH_2) group of (P-Bromo aniline) the absorption band $(1435) \text{ cm}^{-1}$ was due to ($\text{N}=\text{N}$) stretching band of azo group, (NH_2 amine sulfonamide) 3348 , ($\nu \text{ N-H pyrimidine}$) 3252 , ($\nu \text{ C=N pyrimidine}$) 1647 , ($\nu \text{ C=C aromatic}$) 1489 , ($\nu \text{ C-N}$) 1256 , ($\nu \text{ C-S}$) 808 , ($\nu \text{ SO}_2$) $1317, 1146$. All of these absorption bands are another good evidence to formation our azo dyes compound[3].

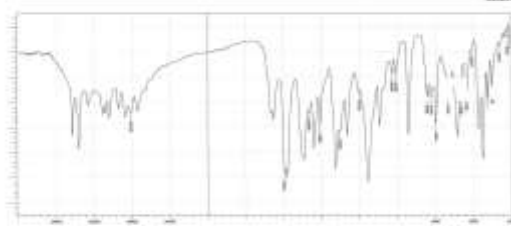
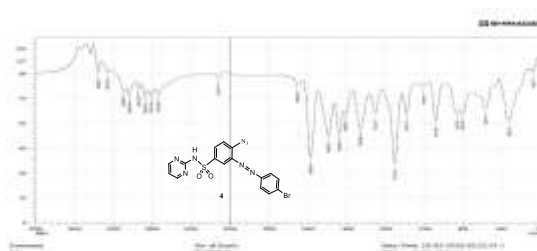
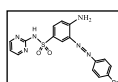


Figure (2): FTIR spectrum of compound [3]

Figure (3): FTIR spectrum of compound [4].



3.3 Compound [4]: Synthesis of new azido sulfa drugs (4) synthesis by reaction [3] with sodium nitrate NaNO_2 and HCl in (0-5 °C) to prepare diazonium salt after that the addition of sodium azide (NaN_3) at same temperature leads to synthesized new azide derivative and identified by disappearance of primary amine in [3] at 3358 cm^{-1} and appearance of special peak at 2119 cm^{-1} , $\nu(\text{NH})$ 3356 , $\nu(\text{CH})_{\text{aliph}}$ 2941 , $\nu(\text{SO}_2_{\text{asy\&sy}})$ $1330, 1153$, $\nu(\text{C-N})$ 1253 , $\nu(\text{N=N})$ 1438 , $\nu(\text{C-S})$ 679 . and other peak as shown in

3.4 Compound [5]: New amide according to $\text{S}_\text{N}2$ mechanism of reaction of [3] with chloroacetyl chloride to synthesis [5]. The FTIR spectrum shows the disappearance of primary amine at 3358 cm^{-1} for starting material [3] and the appearance of new peaks for the carbonyl amide group ($\nu(\text{C=O})$ 1750 cm^{-1} , $\nu(\text{NH})$ 3366 cm^{-1} , $\nu(\text{CH}_{\text{aliph}})$ $2947-2867\text{ cm}^{-1}$, $\nu(\text{C-Cl})$ 677 cm^{-1} .

3.5 Compound [6]: Synthesis of new azido sulfa drug [6] according to $\text{S}_\text{N}2$ mechanism chloride in [5] substituted by azido group (by using NaN_3 in DMSO) to synthesized new azide derivative and identified by disappearance C-Cl band at 677 cm^{-1} in [5] and appearance of new band in 2112 cm^{-1} for new azide group, and other peak ($\nu(\text{NH})$ 3375 , $\nu(\text{CH}_{\text{Ar}})$ 3034 , $\nu(\text{CH}_{\text{aliph}})$ $2924, 2852$, $\nu(\text{C=O})$ 1757 , $\nu(\text{C=C}_{\text{aromatic}})$ 1589 , $\nu(\text{SO}_2_{\text{asy\&sy}})$ $1311, 1155$, $\nu(\text{C-N})$ 1251 , $\nu(\text{N=N})$ 1514 , $\nu(\text{C-S})$ 698 . as shown in FTIR spectrum in figure (4).

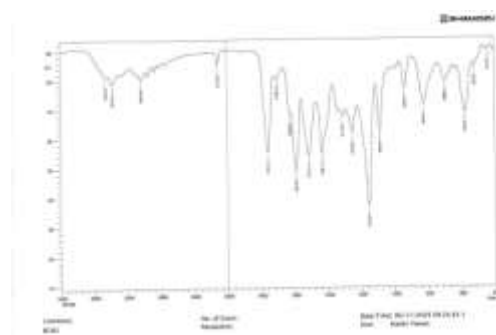
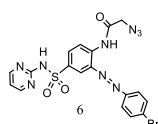


Figure (4): FTIR spectrum of compound [6].

3.6 Compound [A4 and A6] are identified by the disappearance of peak at $2108-2112\text{ cm}^{-1}$ for the azido group and 2119 cm^{-1} for propargylic derivative for starting materials and new peaks appearance as shown in FTIR spectrum for the synthesis of [A4 and A6] in table (1).

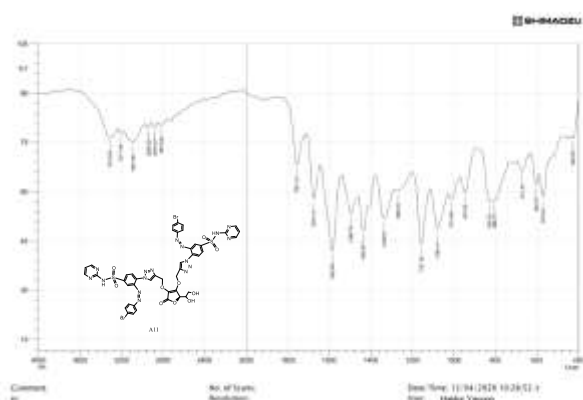
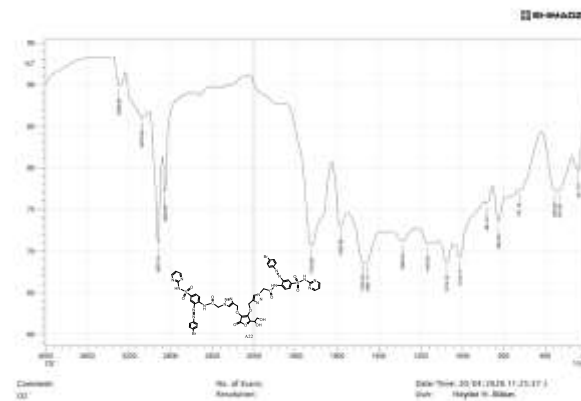
Table(1): FTIR spectrum data of compounds [A4 and A6]

Comp.	$\nu(\text{CH})_{\text{ar.}}$	$\nu(\text{CH})_{\text{aliph}}$	$\nu(\text{C}=\text{C})$	$\nu(\text{NH})$	$\nu(\text{C}-\text{O}-\text{C})$	$\nu(\text{C}=\text{O})$
A4	3028	2922-2868	1591	3275	1089	1662
A6	3028	2941-2872	1593	3414	1251	1658

3.7 Compound [A44 and A66] : General de-protection of compounds Compound [A4 and A6] by using Iodene in Methanol and shown in new peaks appearance as shown in FTIR spectrum figures (5-6) for the synthesis of [A4 and A6] and table (2).

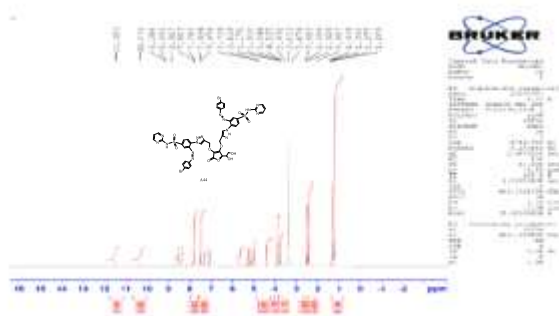
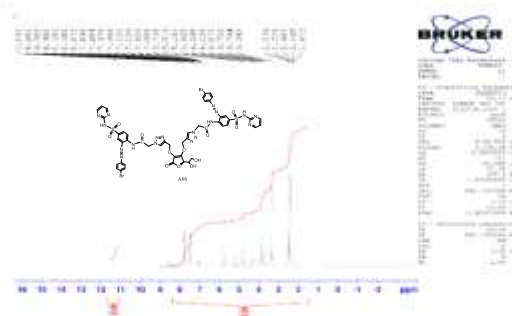
Table(2): FTIR spectrum data of compounds [A44 and A66]

Comp.	$\nu(\text{CH})_{\text{ar.}}$	$\nu(\text{CH})_{\text{aliph}}$	$\nu(\text{C}=\text{C})$	$\nu(\text{C}=\text{O})$	$\nu(\text{OH})$ $\nu(\text{NH})$
A44	3035	2922-2854	1583	1666	3194
A66	3032	2924 2858	1587	-----	3201

**Figure (5):** FTIR spectrum of compound [A44].**Figure (6):** FTIR spectrum of compound [A66].

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) [A44] δppm : 11.66 ($-\text{SO}_2-\text{NH}$), 6.94-7.89($-\text{CH}-\text{Ar}$), 8.08 (CH triazole), 5.34,5.11 (triazole- CH_2-O), 3.35($-\text{CH}_2\text{O}$), 8.48,7.02 ($-\text{CH}$ pyrimidine), 3.94 ,5.77 (diol)

$^1\text{HNMR}$ (400 MHz, DMSO-d_6) [A66] δppm : 7.55 ($-\text{CH}$ triazole), 7.80-8.57($-\text{CH}-\text{Ar}$), 7.55 (triazole: CH_2-), 5.05 ($-\text{CH}_2-\text{C}=\text{O}$) , 5.11,5.34 ($\text{CH}-\text{O}-$), 11.32 ($-\text{SO}_2-\text{NH}$) ,3.94,5.77($-\text{OH}$ diol). figures (7-8) show the $^1\text{HNMR}$ spectrum of this derivatives.

**Figure (7):** $^1\text{HNMR}$ spectrum of compound [A44].**Figure(8):** $^1\text{HNMR}$ spectrum of compound [A66].

$^{13}\text{C-NMR}$ (400 MHz, DMSO-d_6) [A44] δppm :115.3 -159.5($-\text{C}=\text{N}$ pyrimidine),122.2-135.6 ($\text{Ar}-\text{C}$), 122.9,142.3 (Triazole carbons) , 125-167(Ascorbic ring).

¹³C-NMR (400 MHz, DMSO-d₆) [A66] ppm: p156.15 (CO_{amide}), 115-159.5 (–CH pyrimidine), 122.9,142.3 (CH_{triazole}),122.2-135.6 (CH-Ar), 62.2,62.5(CH-O), 135 (CH-O).

3.8 Antioxidant activates

3.8.1- Anti-Oxidant activates

The compound A4(at50µg/mL) exhibited the highest scavenging percentages after 5 and 10 minutes compared to other concentrations and compound. However, A4 maintained a relatively stable and superior antioxidant activity throughout the incubation periods. On the other hand, the results showed that A6 (at25µg/mL) reached its peak scavenging activity at 15 minutes, showing a slight increase in inhibition over time. Overall ,the synthesized triazole derivatives showed significant antioxidant potential, with A4 being the most effective at the higher concentration. Table (3)showed Absorbance and Inhibition % of (A4,A6) at 5, 10, and 15 min

Table (3): Absorbance and Inhibition % of (A4,A6) at 5, 10, and 15 min

Control Absorbance =1.119 λ = 517 nm							
Compounds		After 5 min		After 10 min		After 15 min	
		Sample	%	Sample	%	Sample	%
			Inhibition		Inhibition		Inhibition
A4	50µg/mL	0.298	73.37	0.300	73.19	0.311	72.21
	25µg/mL	0.296	73.55	0.299	73.28	0.301	73.10
A44	50µg/mL	0.294	73.73	0.295	73.64	0.296	73.55
	25µg/mL	0.260	76.76	0.285	74.53	0.277	75.25

Statistical and Graphical Analysis of IC₅₀

The linear regression equations generated from the scatter plots revealed an exceptionally high baseline of antioxidant activity for the tested compounds. Due to the high inhibition percentages (exceeding 70%) achieved at the lowest experimental concentration (25 µg/mL), the trendlines extrapolated backward into the negative region of the X-axis to intercept the 50% inhibition threshold. > Mathematically, this mathematical artifact results in negative or unusually high absolute IC₅₀ values when applying the standard linear equation ($IC_{50} = \frac{50 - a}{b}$). Biologically, these findings confirm that the true IC₅₀ value is well below the lowest tested concentration ($IC_{50} < 25 \mu\text{g/mL}$), demonstrating the outstanding efficacy of the synthesized compounds as potent radical scavengers

Figure (9): The Standard Calibration Curve of (A4 and A6)

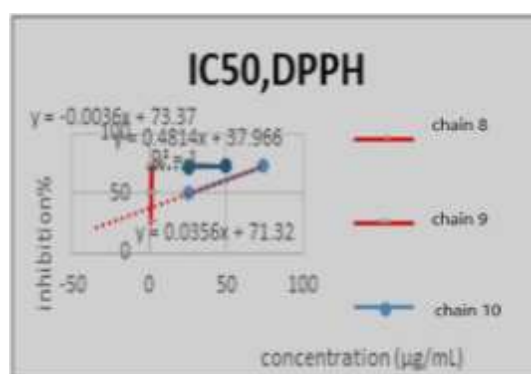


Table (4): Linear Regression and IC₅₀ Values of (A4 and A6) and Ascorbic acid.

Compounds	Linear Regression Equation	R ² Value	IC ₅₀ (µg/mL)
A4(5 min)	y = 0.0356x + 71.32	0.9994	< 25
A4(10 min)	y = -0.0036x + 73.37	1.0000	< 25

A4(15 min)	$y = 0.4814x + 37.966$	0.9991	25.00
A44(5 min)	$y = 0.0396x + 73.181$	0.9999	< 25
A44(10 min)	$y = -0.0536x + 75.601$	0.9998	< 25
A44(15 min)	$y = -0.0036x + 73.37$	1.0000	< 25
Ascorbic acid (Standard)	$y = 0.1654x + 48.122$	0.9885	4.215

4. Conclusion

Novel Azo Sulfadiazine derivatives -1,2,3-triazole –L-Ascorbic Acid molecular hybrids were successfully designed and Synthesized by catalyzed Huisgen 1,3-dipolar cycloaddition (Click Chemistry) between Sulfadiazine azo azide derivatives with Bis -2,3-O –propargyl -5,6-O-isopropylidene L-Ascorbic Acid ,then isopropylidene ring be removed to de protected 5,6-position.

After full spectroscopic Characterization,the newly Synthesized compounds were investigated for their Antioxidant Activity,All Synthesized Ascorbic Acid derivatives that investigated A4,A44, revealed a marked effectiveness in the treatment with DPPH rather than Ascorbic acid ,this may be by a Synergistic Effect between Sulfonamide-1,2,3-triazole and L-Ascorbic Acid that have extended conjugated system which plays a fundamental role in stabilizing free radicals through resonance combine biologically active heterocyclic cores with functional groups capable of scavenging free radicals

Acknowledgments

This article is derived from the forthcoming PhD thesis of Nuha Salman Salih Rabee , Department of Chemistry, Faculty of Science, University of Kufa .

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