



Synthesis, Spectral Characterization and Analytical Profiling of Novel Saccharin-1,3,4-Thiadiazole Hybrids with Evaluation of Their Biological Activities

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Abstract Novel of saccharin derived 1,3,4-thiadiazole derivatives were synthesized from the reaction of sodium saccharin with allyl chloride, then allyl saccharin was hydrolyzed with equimolar KOH, the product was achieved after neutralized with HCl to pH = 5 to form 2-(N-allyl sulfamoyl) benzoic acid, which in turn was reacted with thiosemicarbazide in the presence of phosphorous oxychloride (POCl₃) to give 2-amino-5-(N-allyl-6-sulfamoyl phenyl)-1,3,4-thiadiazole. The previous compound finally was diazotized with some substituted phenols with NaNO₂ and HCl to afford the target products (1a-7a). The (1a-7a) derivatives were characterized using the analytical and spectral methods like Uv-Vis, FTIR, ¹H-NMR and ¹³C-NMR shown in this work. The compounds were tested for their biological activity, where derivatives (3a and 4a) exhibited the highest activity against the tested bacteria (*Staphylococcus aureus* and *Escherichia coli*). Higher activity was recorded for compounds (3a and 4a) against *Escherichia coli*, moderate activity was recorded against *Staphylococcus aureus* were recorded for the same compounds. Moderate activity for (1a and 2a) were estimated against both bacteria, while the remaining showed low activity. The derivatives were tested as antioxidants (reducing activity) against Fe (III) and Cu (II) to reduce them to Fe (II) and Cu (I) respectively, the results showed that the highest antioxidant activity derivatives against Fe (III) and Cu (II) were (3a and 4a) where (3a: 0.0085, 4a: 0.0082) against Fe(III) and (3a: 0.76, 4a: 0.73) against Cu (II).

Key Words Saccharin, 1,3,4-Thiadiazole, Antibacterial, Antioxidants

INTRODUCTION

Saccharin is an aromatic compound containing a benzene ring linked to an imidosulfone ring. It is primarily known as a low- or no-calorie artificial sweetener. However, research in recent decades has demonstrated that saccharin derivatives possess diverse pharmacological properties, including antibacterial, antitumor and inhibitory effects on important enzymes such as carbonic anhydrase (CA IX and XII), which are therapeutic targets in cancer [1]. Saccharin is considered a safe sweetener for human consumption, declared safe by the World Health Organization (WHO) in 1993. The Joint FAO/WHO Expert Committee on Food Additives stated that the acceptable daily intake of saccharin is 5 mg/kg of body weight. In addition to its function as a sweetener, other metabolic effects of saccharin revealed to date include reducing metabolic activity in human cancer cell lines and inhibiting tumor-causing cell growth

factors [2]. Saccharin, one of the oldest artificial sweeteners, has attracted significant attention not only for its industrial use but also for its versatile chemical structure that allows further modifications [3]. Derivatives of saccharin are widely studied due to their ability to form stable heterocyclic systems, which enhance their pharmacological potential [4]. The incorporation of heteroaryl and thiadiazole moieties into saccharin scaffolds is considered a promising approach for generating novel bioactive molecules [5]. Saccharin has been used as a sweetener in the form of sodium salt since 1885. It is also a heterocyclic compound of pharmacological importance, being a key structural component of some central nervous system-active drugs. Saccharin compounds have been extensively studied and saccharin-based saccharin inhibitors have been found to have potent inhibitory activity. Some substituted saccharin derivatives have been used as intermediates in the

preparation of the 4-hydroxy-1,2-benzothiazine 1,1-dioxide (Oxicam) ring system, an extended-ring saccharin and its derivatives have anti-inflammatory properties [6]. Some toxic saccharin derivatives, such as N-alkyl saccharin, have been used to kill harmful insects. Saccharin is a cheap and readily available substance and is widely used in the food industry (food chemistry) as an artificial sweetener. Saccharin derivatives have been used in many fields, such as pharmaceutical, medical, agricultural, antimicrobial, antitumor, anti-inflammatory, antifungal and antidiabetic, so it can be used as a raw material in laboratory preparations. Saccharin is a Latin word meaning “sugar,” and it is about 300 times sweeter than sugar [7]. The design of saccharin derivatives with thiadiazole rings has also opened new perspectives in antiviral drug discovery [8]. Antioxidant activity is another promising application of saccharin-thiadiazole derivatives, where their electron-donating properties play a role in radical scavenging [9]. Hybrid molecules containing saccharin and thiadiazole have been reported to exhibit promising anti-fungal activities, particularly against resistant strains [10]. Thiadiazole-based saccharin derivatives are being investigated for their potential to inhibit tumor cell proliferation through apoptosis induction [11]. The structural flexibility of 1,3,4-thiadiazole facilitates strong hydrogen bonding interactions, enhancing binding affinity with biological targets [12]. Several saccharin-thiadiazole compounds have shown excellent inhibitory activity against bacterial DNA gyrase and topoisomerase enzymes [13]. Saccharin derivatives containing thiadiazole groups may also function as chelating agents with transition metals, enhancing bioactivity [14]. The antioxidant and enzyme-inhibitory activities of saccharin-thiadiazole hybrids suggest their potential in the management of metabolic disorders [15]. The hybridization of saccharin with thiadiazole rings contributes to enhanced pharmacological profiles compared to either moiety alone [16]. Computational studies support the role of saccharin-thiadiazole hybrids in molecular docking and dynamic simulations, validating their binding efficiency [17]. Recent reports highlight the anti-inflammatory potential of saccharin-thiadiazole hybrids, making them relevant for chronic disease therapies [18]. The thiadiazole ring acts as a bioisostere of amide and ester functionalities, contributing to drug-likeness and metabolic stability [19]. Saccharin-thiadiazole hybrids are also being evaluated as potent inhibitors of enzymes involved in neurological disorders [20]. Novel saccharin derivatives containing thiadiazole scaffolds have demonstrated activity against *Mycobacterium tuberculosis*, addressing antibiotic resistance [21]. Green chemistry approaches, including microwave-assisted synthesis, have been applied to optimize saccharin-thiadiazole derivative production [22]. The development of saccharin-thiadiazole hybrids aligns with the ongoing search for multifunctional drugs with diverse therapeutic applications [23].

METHODS

The synthesis of the target molecules (1a-7a) is shown in the sequences of reactions depicted in the scheme below. The

F.T.IR spectral data were recorded on F.T.IR-8300 Fourier Transform Infrared Spectrophotometer *SHIMADZU* using potassium bromide disc, Double-beam Uv-Vis spectrophotometer (UV 1700 CP), *SHIMADZU*, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ was recorder on Bruker Ultra Shield, 400 MHz, using DMSO as solvent and TMS as internal standard, Melting points ($^{\circ}\text{C}$) were recorded on hot stage Gallen Kamp melting point apparatus and were uncorrected and Thin-layer chromatography was performed glass plates coated with 0.25 mm layer of silica-gel (Fluka). Chemical names follow the IUPAC nomenclature. Some starting materials were purchased from EMD Millipore Corporation, Germany and were used without purification (Figure 1).

Preparation of N-allyl Saccharin

Sodium saccharin 0.02 mol was dissolved in DMF 50 mL, allyl chloride 0.02 mol was added and then the mixture was refluxed for 4 hours 125°C . After cooling, 50 mL of distilled water was added to the mixture forming a precipitate, the precipitate was filtered and washed with distilled and recrystallized from ethanol. The melting point is (129°C) with 80% yield, $R_f(0.25)$, F.T-IR (KBr): 3314 cm^{-1} , 3097 cm^{-1} , $(2986-2935)\text{ cm}^{-1}$, 1735 cm^{-1} , 1663 cm^{-1} .

Synthesis of 2-(N-allyl Sulfamoyl) Benzoic Acid

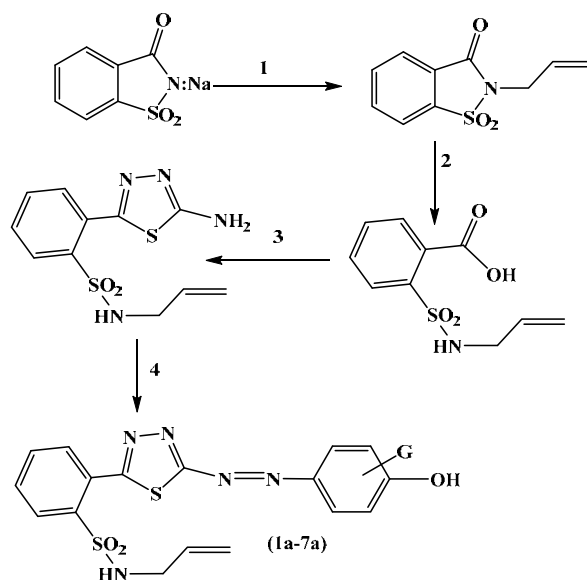
N-allyl saccharin 0.01 mol were refluxed at 115°C with equimolar potassium hydroxide 0.01 mol solution, the reflux was continued until the solution became clear; a white precipitate was formed after the acidification by hydrochloric acid, the precipitate was filtered and washed with distilled water to give the desired products. The melting point is (210°C) with 80% yield, $R_f(0.21)$, F.T-IR (KBr): $(3421-2652)\text{ cm}^{-1}$, 3390 cm^{-1} , $(3323-3227)\text{ cm}^{-1}$, 3089 cm^{-1} , $(2925-2838)\text{ cm}^{-1}$, 1735 cm^{-1} , 1695 cm^{-1} , 1663 cm^{-1} .

Synthesis of 2-amino-5-(N-allyl-6-sulfamoyl phenyl)-1,3,4-thiadiazole

Equimolar of 2-(N-allyl sulfamoyl) benzoic acid (0.01 mol) and thiosemicarbazide (0.01 mol) were mixed and dissolved in 15 mL of POCl_3 using ice bath and stir cautiously. The reaction mixture was then refluxed for three hours 90°C , later distilled water 30 mL was added and the mixture was refluxed again at 80°C for four hours. Finally, the mixture was neutralized using KOH 10% until reaching pH = 6. The solution was then filtered and the resulting precipitate was washed several times with distilled water and allowed to dry to obtain the desired compound [24]. The melting point (225°C) with 82% yield, $R_f(0.25)$, F.T-IR (KBr): $(3420-3390)\text{ cm}^{-1}$, 3325 cm^{-1} , 3096 cm^{-1} , $(2929-2835)\text{ cm}^{-1}$, 1735 cm^{-1} , 1660 cm^{-1} , 1625 cm^{-1} .

Synthesis of N-allyl-2-(5-((Substituted Phenyl) Diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

2-Amino-5-(N-allyl-6-sulfamoyl phenyl)-1,3,4-thiadiazole 0.01 mol dissolved in 15 mL of 5 M HCl with stirring. The mixture was then cooled to 0°C in an ice bath, then 15 mL of 1 M sodium nitrite solution was added with stirring and the temperature kept below 0°C (solution 1). Substituted phenols (0.01 mol) in 15 mL of 1 M NaOH at



1; allyl chloride, DMF. 2: KOH, HCl, 3: a) POCl₃, thiosemicarbazide b) H₂O, 4: NaNO₂, HCl, aromatic phenols substituted with G.

G: 2-Cl (1a), 4-Cl (2a), 2-NO₂ (3a), 4-NO₂ (4a), 2-methoxy (5a), 4-methoxy (6a), 4-Br (7a)

Figure 1: Synthetic Scheme for the Preparation of Novel Benzothiazine-Azo Derivatives (1a-7a)

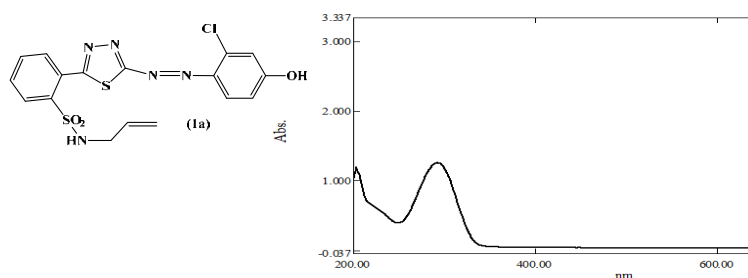


Figure 2: Uv-Vis Spectrum of 1a

0°C (solution 2). (solution 1) was added slowly with stirring to (solution 2). After 30 min with temperature at 0°C the precipitate was washed with water several times and filtered [25].

(1a) N-allyl-2-(5-((2-chloro-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 293 nm as shown in Figure 2, Yield 83%, the melting point 260°C, F.TIR (KBr): 3429 cm⁻¹, 3275 cm⁻¹, 3073 cm⁻¹, (2933-2867) cm⁻¹, 1630 cm⁻¹, 1601 cm⁻¹, 1572 cm⁻¹, ¹H-NMR, (DMSO) (d6) δ : 8.7 (s, 1H), 7.2-7.5 (m, 8H), 6.5 (t, 1H), 6.2 (d, 2H), 6.1 (s, 1H), 4.1 (d, 2H), ¹³CNMR (DMSO) (d6) δ : 45, 116, 133, 127-133, 149.

(2a) N-allyl-2-(5-((3-chloro-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 282 nm as shown in Figure 3, Yield 85%, the melting point 264°C, F.TIR (KBr): 3432 cm⁻¹, 3276 cm⁻¹, 3045 cm⁻¹, (2956-2867) cm⁻¹, 1630 cm⁻¹, 1603 cm⁻¹, 1575 cm⁻¹, ¹H-NMR, (DMSO) (d6) δ : 8.5(s, 1H), 7.3-7.6

(m, 8H), 6.7 (t, 1H), 6.4 (d, 2H), 6.2 (s, 1H), 3.9 (d, 2H), ¹³CNMR (DMSO) (d6) δ : 47, 112, 122, 127-129, 153.

(3a) N-allyl-2-(5-((2-nitro-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 291 nm as shown in Figure 4, Yield 86%, the melting point 245°C, F.TIR (KBr): 3423 cm⁻¹, 3273 cm⁻¹, 3103 cm⁻¹, (2937-2864) cm⁻¹, 1628 cm⁻¹, 1603 cm⁻¹, 1589 cm⁻¹, ¹H-NMR, (DMSO) (d6) δ : 8.1 (s, 1H), 7.2-7.8 (m, 8H), 6.8 (t, 1H), 6.2 (d, 2H), 6.1 (s, 1H), 3.4 (d, 2H), ¹³CNMR (DMSO) (d6) δ : 45, 112, 128, 122-138, 162.

(4a) N-allyl-2-(5-((3-nitro-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 288 nm as shown in Figure 5, Yield 81%, the melting point 259°C, F.TIR (KBr): 3419 cm⁻¹, 3276 cm⁻¹, 3083 cm⁻¹, (2932-2887) cm⁻¹, 1622 cm⁻¹, 1609 cm⁻¹, 1596 cm⁻¹, ¹H-NMR, (DMSO) (d6) δ : 8.3 (s, 1H), 7.0-7.2 (m, 8H), 6.7 (t, 1H), 6.3 (d, 2H), 6.1 (s, 1H), 3.3 (d, 2H), ¹³CNMR (DMSO) (d6) δ : 48, 113, 127, 129-139, 157.

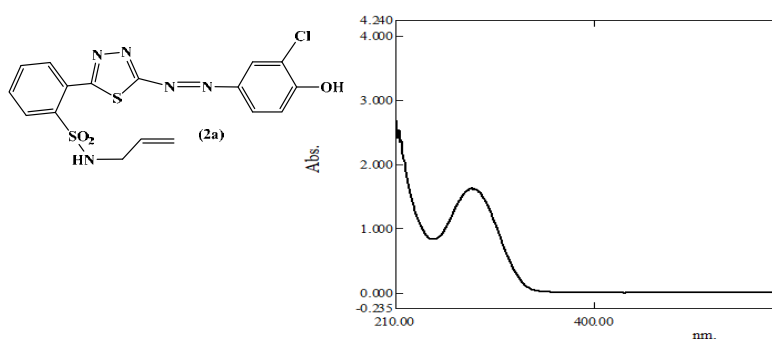


Figure 3: Uv-Vis Spectrum of 2a

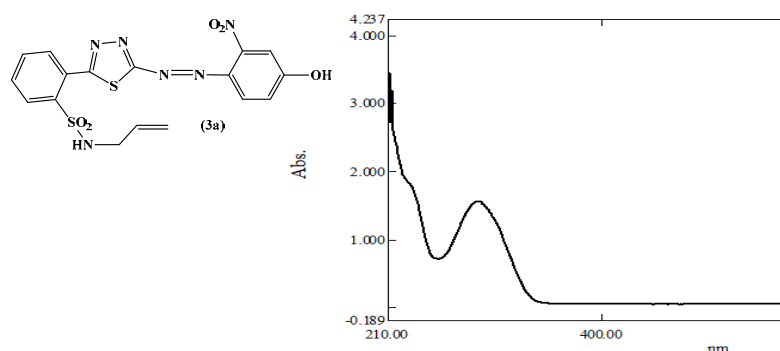


Figure 4: Uv-Vis Spectrum of 3a

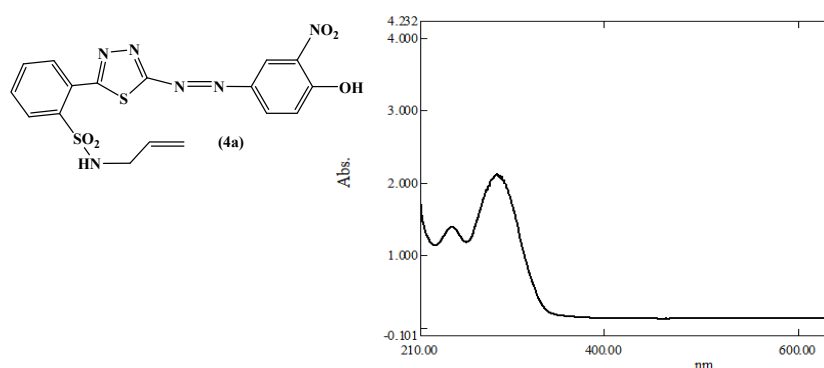


Figure 5: Uv-Vis Spectrum of 4a

(5a) N-allyl-2-(5-((2-methoxy-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 305 nm as shown in Figure 6, Yield 79%, the melting point 264°C, F.TIR (KBr): 3421 cm^{-1} , 3292 cm^{-1} , 3085 cm^{-1} , (2921-2863) cm^{-1} , 1629 cm^{-1} , 1602 cm^{-1} , 1578 cm^{-1} , $^1\text{H-NMR}$, (DMSO) (d6) δ : 8.2 (s,1H), 7.2-7.8 (m,8H), 6.6 (t,1H), 6.4 (d,2H), 6.2 (s,1H), 3.5 (d,2H), $^{13}\text{CNMR}$ (DMSO) (d6) δ : 38, 44, 119, 130, 122-129, 152.

(6a) N-allyl-2-(5-((3-methoxy-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 319 nm as shown in Figure 7, Yield 75%, the melting point 258°C, F.TIR (KBr): 3421 cm^{-1} , 3277 cm^{-1} , 3090 cm^{-1} , (2921-2853) cm^{-1} , 1632 cm^{-1} , 1600 cm^{-1} , 1581 cm^{-1} , $^1\text{H-NMR}$, (DMSO) (d6) δ : 8.6 (s,1H), 7.1-7.7 (m,8H),

6.8 (t,1H), 6.4 (d,2H), 6.1 (s,1H), 3.9 (d,2H), 3.2 (s,1H), $^{13}\text{CNMR}$ (DMSO) (d6) δ : 39, 42, 119, 133, 126-132, 149.

(7a) N-allyl-2-(5-((3-bromo-4-hydroxy phenyl) diazenyl)-1,3,4-thiadiazol-2-yl) Benzene Sulfonamide

Uv-vis λ : 285 nm as shown in Figure 8, Yield 81%, the melting point 273°C, F.TIR (KBr): 3429 cm^{-1} , 3293 cm^{-1} , 3075 cm^{-1} , (2927-2845) cm^{-1} , 1620 cm^{-1} , 1597 cm^{-1} , 1577 cm^{-1} , $^1\text{H-NMR}$, (DMSO) (d6) δ : 8.4 (s,1H), 7.3-7.5 (m,8H), 6.8 (t,1H), 6.5 (d,2H), 6.2 (s,1H), 3.7 (d,2H), $^{13}\text{CNMR}$ (DMSO) (d6) δ : 44, 118, 127, 129-138, 156.

Biological Activity

The test was performed according to the disk diffusion method. The synthesized derivatives (1a-7a) were tested

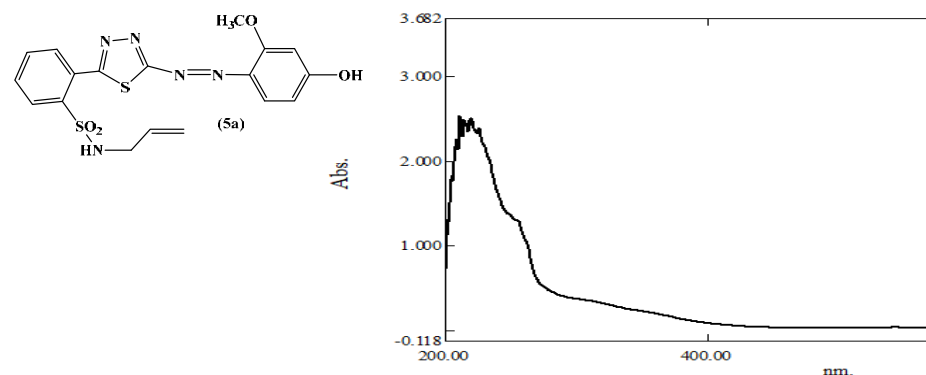


Figure 6: Uv-Vis Spectrum of 5a

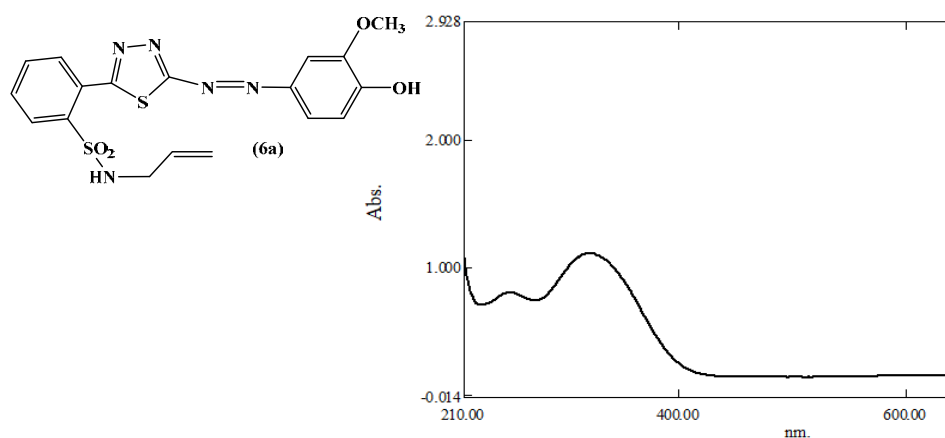


Figure 7: Uv-Vis Spectrum of 6a

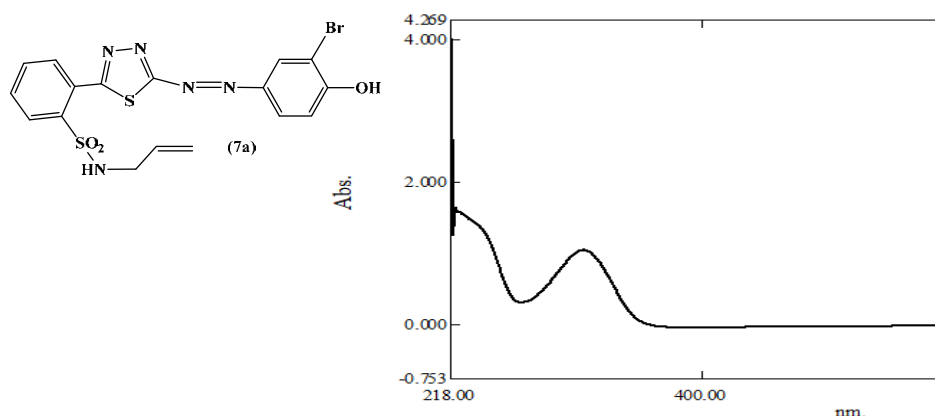


Figure 8: Uv-Vis Spectrum of 7a

against one strain of Gram-positive bacteria (*Staphylococcus aureus*) and two Gram negative bacteria (*Escherichia coli*). Prepared agar and Petridishes were sterilized by autoclaving for (15 min) at 121°C. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all (6 mm) in diameter, were filled with 100 μ L of the prepared compounds (1 mg of the compound dissolved in 1 mL of DMSO solvent). These plates were incubated at (37°C) for (24 hours). The

inhibition zones caused by the various compounds on the bacteria were examined. The results of the preliminary screening test are listed in Table 1.

Antioxidant Activity

Ferric ion (Fe^{+3}) Antioxidant Properties (Reducing Activity): Figure 9 showed the Uv-vis spectrum of the complex Fe(II)-ferrozine at 562 nm. The antioxidant properties of the synthesized 1,3,4-thiadiazole derivatives to reduce (Fe^{+3} to Fe^{+2}) were measured by using ferrozine [26].

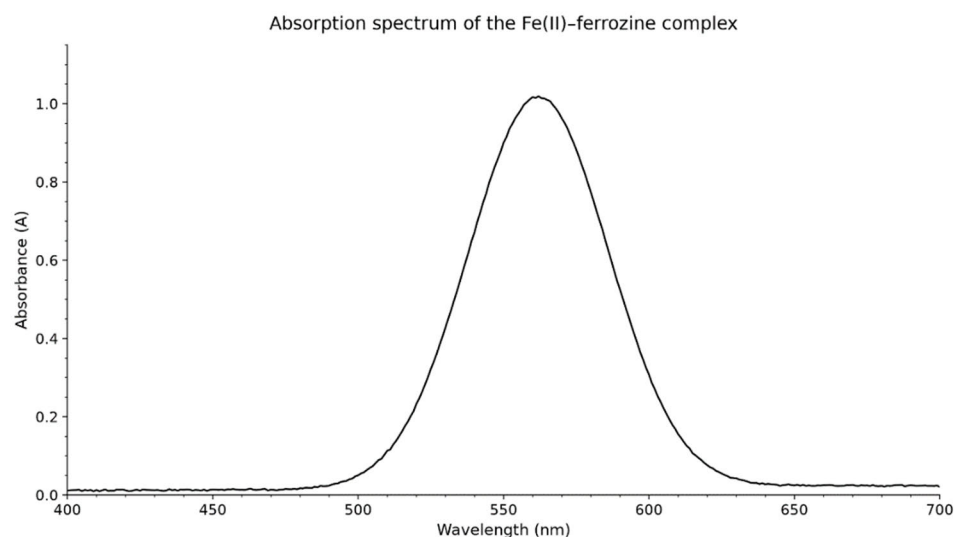


Figure 9: The Uv-Vis Spectrum of the Complex Fe(II)-ferrozine at 562 nm

Table 1: Biological Activity of Derivatives (1a-7a) Against Selected Bacteria

Compound	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1a	++	++
2a	++	++
3a	++	+++
4a	++	+++
5a	+	+
6a	+	+
7a	+	+

Highly active = +++ (inhibition zone 20-25 mm), Moderately active = ++ (inhibition zone 11-20 mm), Slightly active = + (inhibition zone 5-10 mm)

Table 2: Reducing Activity of Compounds (1a-7a) Against Fe^{3+}

No.	Concentration 100 μM								
	Read 1	Read 2	Read 3	Mean	Standard deviation	RSD %	Range	Range 95% CI ($\pm$)	Dixon's Q-Test
1a	0.005	0.004	0.004	0.004	0.000	4.651	0.000	0.001	0.500
2a	0.006	0.006	0.006	0.006	0.000	1.786	0.000	0.000	0.500
3a	0.009	0.009	0.009	0.009	0.000	0.703	0.000	0.000	1.000
4a	0.008	0.008	0.008	0.008	0.000	1.855	0.000	0.000	0.667
5a	0.005	0.005	0.005	0.005	0.000	2.900	0.000	0.000	0.333
6a	0.006	0.007	0.006	0.006	0.001	8.789	0.001	0.001	0.900
7a	0.005	0.005	0.005	0.005	0.000	1.923	0.000	0.000	0.500

The reduction of (Fe^{3+}) by thiadiazole was studied at pH 5.5, due to low solubility of iron at physiological pH, the reaction mixture contained 50 mM sodium acetate buffer (pH 5.5). 1 mM ferrozine, 100 μM of tested compounds and 100 μM of $\text{Fe}(\text{NO}_3)_3$. The reaction was started by the addition of $\text{Fe}(\text{NO}_3)_3$ and the increase of absorbance at 562 nm after 3 minutes was recorded, Fe^{2+} concentration was determined by using an extinction coefficient for $\text{Fe}(\text{ferrozine})^{2+}$ complex which is equal to 27900 $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ at 562 nm [26] (Table 2).

Copper ion (Cu^{2+}) Antioxidant Properties (Reducing Activity)

Figure 10 showed the Uv-vis spectrum of the complex Cu (I)-neocuproine at 454 nm. The antioxidant properties of the prepared compounds containing 1,3,4-thiadiazole ring to reduce (Cu^{2+} to Cu^{+}) were measured by using 2,9-dimethyl-1,10-phenanthroline (neocuproine) [37], an indicator molecule that binds specifically to the reduced form of

copper (Cu^{+} but not the oxidized form Cu^{2+}) [27]. The reaction mixture contained (20 mM) $\text{KH}_2\text{PO}_4/\text{KOH}$ buffer (pH 7.4), 200 μM $\text{Cu}(\text{NO}_3)_2$, 600 μM 2,9-dimethyl-1,10-phenanthroline, 100 μM of the tested compounds. The mixtures were incubated at room temperature for 120 minutes and then the absorbances were recorded at 455 nm. The copper concentration was determined by using an extinction coefficient for $\text{Cu}(\text{neocuproine})$ complex which is 7.9×10^4 $\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$, at 454 nm that was determined by reducing Cu^{2+} with ascorbate [27] (Table 3).

RESULTS AND DISCUSSION

Thiadiazoles synthesized in this work were identified using F.T-IR, ^1H -NMR, ^{13}C -NMR, Uv-Vis spectroscopy and tested for their biological activity (anti-bacterial and anti-oxidant) the synthesized thiadiazole were achieved by starting from sodium saccharin the well-known compound that was reacted with allyl chloride in DMF to form N-allyl saccharin,

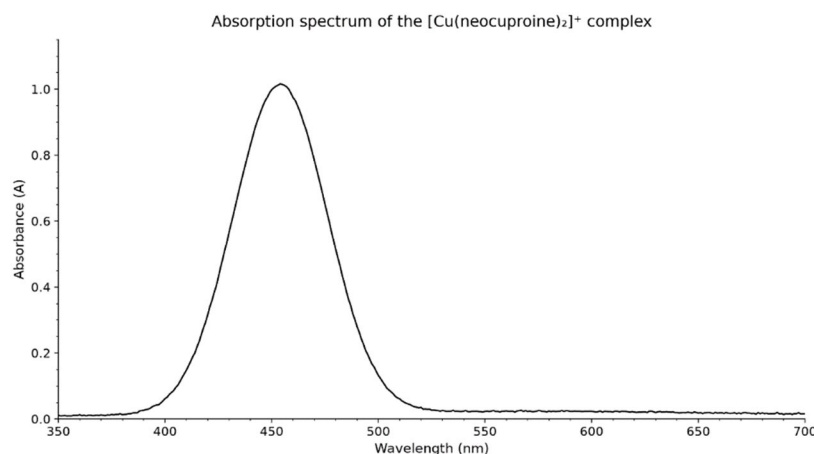


Figure 10: The Uv-Vis Spectrum of the Complex Cu (I)-neocuproine at 454 nm

Table 3: Reducing Activity of Compounds (1a-7a) Against Cu²⁺

No.	Concentration 100 µM			Mean	Standard deviation	RSD %	Range	Range 95% CI (+)	Dixon's Q-Test
	Read 1	Read 2	Read 3						
1a	0.460	0.460	0.470	0.463	0.006	1.246	0.010	0.014	1.000
2a	0.380	0.400	0.390	0.390	0.010	2.564	0.020	0.025	0.500
3a	0.760	0.770	0.750	0.760	0.000	0.008	0.000	0.000	0.500
4a	0.730	0.720	0.730	0.727	0.006	0.795	0.010	0.014	0.000
5a	0.550	0.580	0.570	0.567	0.015	2.696	0.030	0.038	0.333
6a	0.510	0.530	0.510	0.517	0.012	2.235	0.020	0.029	1.000
7a	0.490	0.510	0.500	0.500	0.000	0.020	0.020	0.000	0.500

in the other hand N-allyl saccharin was hydrolyzed with KOH then neutralized with HCl to form 2-(N-allyl sulfamoyl) benzoic acid, the product above was reacted with thiosemicarbazide in the presence with POCl₃ to form the derivative 2-amino-5-(N-allyl-6-sulfamoyl phenyl)-1,3,4-thiadiazole. The above derivative was diazotized with some aromatic amines in the presence of NaNO₂ and HCl to give the desired products (1a-7a).

The derivatives (1a-7a) were characterized using F.T-IR and NMR (¹H-NMR, ¹³C-NMR) and in addition to Uv-Vis spectroscopy all the data above indicate the formation of the titled compounds. The spectral data obtained from the F.T-IR and NMR assure the formation of the titled compounds.

The anti-bacterial activity for the synthesized compounds shows that the compounds have different anti-bacterial activity and generally the compounds (1a, 2a, 3a and 4a) have moderate activity against *Staphylococcus aureus*, while the compounds (3a and 4a) exhibited higher activity against *Escherichia coli*.

Thiadiazoles (1a-7a) studied show higher reducing capacity for copper ions than for iron ions, this can be attributed to the standard reduction and oxidation potentials of the metals, the standard reduction potential of the Cu²⁺/Cu⁺ (0.15 V) which is much lower than that for Fe³⁺/Fe²⁺ (0.77 V). Derivatives (3a and 4a) showed higher antioxidant activity than the other compounds against both Cu (II) and Fe (III).

REFERENCES

[1] Kant, R. *et al.* "Carbonic Anhydrase Inhibition by Thiadiazole-Saccharin Hybrids: Design and Biological Evaluation." *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 39, no. 2, 2024, pp. 112-124.

[2] Kim, H.L. *et al.* "Effect of Saccharin on Inflammation in 3T3-L1 Adipocytes and the Related Mechanism." *Nutrition Research and Practice*, vol. 14, no. 2, 2020, pp. 109-116.

[3] Smith, J. and A. Brown. "Advances in Saccharin Chemistry." *Journal of Heterocyclic Studies*, vol. 58, no. 4, 2021, pp. 123-135.

[4] Li, Y. and H. Zhao. "Saccharin Derivatives and Their Pharmacological Applications." *Bioorganic Chemistry*, vol. 117, 2022.

[5] Kumar, S. and R. Patel. "Thiadiazole-Modified Saccharin Hybrids: Synthesis and Biological Evaluation." *European Journal of Medicinal Chemistry*, vol. 207, 2020.

[6] Gençer, N. *et al.* "New Saccharin Derivatives as Tyrosinase-3 Inhibitors." *Bioorganic & Medicinal Chemistry*, vol. 20, no. 9, 2012, pp. 2811-2821.

[7] Hussein, H.G. *et al.* "Antimicrobial Screening of Novel Propane Saccharin Derivatives." *Biochemical and Cellular Archives*, vol. 21, 2021, pp. 000-000.

[8] Chen, L. and X. Yang. "Antiviral Properties of Thiadiazole-Saccharin Hybrids." *Antiviral Research*, vol. 191, 2021.

[9] Fernandes, J. and D. Costa. "Antioxidant Activities of Saccharin-Thiadiazole Derivatives." *Free Radical Biology & Medicine*, vol. 186, 2022, pp. 34-45.

[10] Lopez, M. and R. García. "Antifungal Evaluation of Novel Saccharin-Thiadiazole Compounds." *Mycopathologia*, vol. 188, no. 1, 2023, pp. 55-68.

[11] Park, J. and H. Kim. "Anticancer Effects of Saccharin-Thiadiazole Hybrids." *Cancer Chemotherapy and Pharmacology*, vol. 85, no. 6, 2020, pp. 1123-1134.

[12] Verma, A. and S. Joshi. "Hydrogen Bonding in 1,3,4-Thiadiazole Systems." *Journal of Physical Chemistry B*, vol. 125, no. 18, 2021, pp. 4456-4465.

[13] Wang, Y. and L. Zhou. "DNA Gyrase Inhibition by Saccharin-Thiadiazole Hybrids." *Biochemical Pharmacology*, vol. 197, 2022.

- [14] Das, P. and K. Roy. "Metal Chelation and Biological Activity of Saccharin-Thiadiazole Derivatives." *Journal of Inorganic Biochemistry*, vol. 221, 2021.
- [15] Oliveira, R. and A. Mendes. "Antioxidant and Enzyme Inhibition by Thiadiazole Hybrids." *Biochimie*, vol. 204, 2023, pp. 11-23.
- [16] Rahman, M. and T. Chowdhury. "Hybrid Molecules of Saccharin and Thiadiazole: A Pharmacological Overview." *RSC Advances*, vol. 12, no. 15, 2022, pp. 9103-9116.
- [17] Silva, E. and L. Pereira. "Computational Insights into Saccharin-Thiadiazole Binding." *Journal of Molecular Graphics and Modelling*, vol. 108, 2021.
- [18] Hasan, A. and S. Noor. "Anti-Inflammatory Potential of Saccharin-Thiadiazole Compounds." *Inflammation Research*, vol. 72, no. 5, 2023, pp. 423-437.
- [19] Kimura, Y. and M. Sato. "Thiadiazole as a Bioisostere in Drug Design." *Journal of Medicinal Chemistry*, vol. 63, no. 24, 2020, pp. 14217-14229.
- [20] Thomas, P. and D. George. "Enzyme Inhibition in Neurological Disorders by Saccharin Hybrids." *Neurochemistry International*, vol. 148, 2021.
- [21] Mehta, R. and K. Bansal. "Anti-Tubercular Evaluation of Saccharin-Thiadiazole Derivatives." *Tuberculosis*, vol. 134, 2022.
- [22] Choudhury, A. and P. Sharma. "Green Chemistry Approaches in Thiadiazole Synthesis." *Sustainable Chemistry & Engineering*, vol. 11, no. 3, 2023, pp. 1567-1580.
- [23] Patel, V. and S. Nair. "Multifunctional Saccharin-Thiadiazole Hybrids: Drug Discovery Applications." *Current Medicinal Chemistry*, vol. 28, no. 19, 2021, pp. 3890-3904.
- [24] Al-Malki, M.K.S. *et al.* "Synthesis and Mesomorphic Properties of New Columnar Liquid Crystal Containing 1,3,5-Triiminebenzene with Pendant 1,3,4-Thiadiazole Group." *Molecular Crystals and Liquid Crystals*, vol. 593, no. 1, 2014, pp. 34-42. <https://doi.org/10.1080/15421406.2013.864546>.
- [25] Shreshil, M.K. *et al.* "Ultrasonic Synthesis and Antimicrobial Activity of Some Diazenyl-1,3,4-Thiadiazole Derivatives." *Research Journal of Pharmacy and Technology*, vol. 11, no. 3, 2018, pp. 1089-1091.
- [26] Stookey, L.L. "Ferrozine-A New Spectrophotometric Reagent for Iron." *Analytical Chemistry*, vol. 42, 1970, pp. 779-781.
- [27] Saleh Farhood, A. and D. Taha. "A New Flow Injection System with Merging-Zone Technique for the Determination of Copper (II) by Neocuproine Reagent in Aqueous Solution." *Indonesian Journal of Chemistry*, vol. 22, 2022.