# Synthesis, antioxidant and hypoglycemic

## assessment of new azo-sulfamethoxazole derivatives

*Síntesis, Evaluación Antioxidante e Hipoglucémica de Nuevos Derivados de Azo-Sulfametoxazol*

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**Abstract**

The link connecting oxidative stress and diabetes is unambiguous. From this perspective, this work was conducted to utilize sulfamethoxazole (**SMZ**) as a precursor to synthesize eight-based derivatives abbreviated as **SMZ 1-8**. Structural identification, carried out using the traditional spectroscopies, namely FTIR, $^{1}$H NMR, and $^{13}$C NMR, was ascertained the chemical frameworks of the synthesized derivatives. The antioxidant and hypoglycemic impacts of these derivatives were investigated to evaluate their potential as antidiabetic candidates. The antioxidant impact was assessed by following the capacity of the synthesized **SMZ** derivatives to track down DPPH- and hydroxyl-reactive species and subscribe an electron in a redox reaction. The hypoglycemic impact was evaluated *in vitro* versus yeast α-glucosidase and porcine α-amylase. The findings indicated that substituting the phenolic azo group for the primary amine moiety of **SMZ** caused a significant increase in the antioxidant and hypoglycemic effects. Also, the synthesized **SMZ** derivatives showed a similar style in their inhibitory efficacy throughout the employed assays. Additionally, **SMZ-2** and **SMZ-6** exhibited delightful antioxidant and hypoglycemic impacts that are exceedingly related to the applied standards. The authors stated that the synthesized **SMZ** derivatives, notably **SMZ-2** and **SMZ-6**, were intriguing candidates for diabetes management and control due to their potent antioxidant and hypoglycemic activities.

**Keywords:** Sulfamethoxazole, Azo, Antioxidant, Hypoglycemic, Diabetes.

**Resumen**

El vínculo que conecta el estrés oxidativo y la diabetes es inequívoco. Desde esta perspectiva, este trabajo se llevó a cabo para utilizar sulfametoxazol (SMZ) como precursor para sintetizar derivados basados en ocho abreviados como SMZ 1-8. La identificación estructural, realizada utilizando las espectroscopias tradicionales, a saber, FTIR, 1H NMR y 13C NMR, permitió determinar las estructuras químicas de los derivados sintetizados. Se investigaron los impactos antioxidantes e hipoglucemiantes de estos derivados para evaluar su potencial como candidatos antidiabéticos. El impacto antioxidante se evaluó siguiendo la capacidad de los derivados de SMZ sintetizados para rastrear especies reactivas de DPPH e hidroxilo y suscribir un electrón en una reacción redox. El impacto hipoglucemiante se evaluó in vitro frente a α-glucosidasa de levadura y α-amilasa porcina. Los hallazgos indicaron que la sustitución del grupo amino primario de SMZ por el grupo azo fenólico provocó un aumento significativo en los efectos antioxidantes e hipoglucemiantes. Además, los derivados de SMZ sintetizados mostraron un estilo similar en su eficacia inhibidora a lo largo de los ensayos empleados. Además, SMZ-2 y SMZ-6 exhibieron efectos antioxidantes e hipoglucemiantes deliciosos que están muy relacionados con los estándares aplicados. Los autores afirmaron que los derivados de SMZ sintetizados, en particular SMZ-2 y SMZ-6, eran candidatos intrigantes para el manejo y control de la diabetes debido a sus potentes actividades antioxidantes e hipoglucemiantes.

**Palabras clave:** Sulfametoxazol, Azo, Antioxidante, Hipoglucemiante, Diabetes.

## Introduction

Oxidative stress is an unavoidable side effect of living in a high-oxygen environment. As a result of aerobic metabolism and exposure to a variety of natural and synthetic toxins, oxygen radicals and other activated oxygen species are produced, which can damage proteins, nucleic acids, and cell membranes[1]. Furthermore, there is emerging evidence that the cumulative damage produced by reactive oxygen species contributes to the development of a wide range of disorders[2].

Antioxidants, which are chemicals that prevent biomolecules, such as nucleic acids, carbohydrates, and proteins, from oxidizing in the presence of low concentrations of free radicals and serve to protect people from free radical damage, have been a major study area in recent years [3]. Synthetic antioxidants are generally phenolic substances that bind to free radicals and inhibit their block-chain reactions. Compared with natural antioxidants, synthetic ones are simple to use, dependable, affordable, long-lasting, and portable. Furthermore, even at modest dosages, they are extremely active[4].

While most diabetes treatments are aimed at managing hyperglycemia, decreasing oxidative stress has been identified as an effective method of handling diabetes and its consequences[5]. The efficiency of this method is supported by increased oxidative damage to biomolecules, decreased antioxidative defense mechanisms in diabetic patients, as well as the potential of several antioxidants employed as adjuvants in the treatment of Type 2 diabetes[6]. Besides, there is a mass of evidence indicating that oxidative stress is, at least partially responsible for the pathogenesis of Type 2 diabetes[7]. However, it is uncertain whether oxidative stress is the main cause, a co-occurring event, or a byproduct of diabetes development[8].

Azo compounds are an important group of chemical molecules with several uses[9]. The relevance of *in vitro* and *in silico* biological research on these compounds is expanding by the day, making a substantial contribution to science[10,11]. The functional group (-N=N-) is formed by uniting two symmetrical and/or asymmetrical chemical entities using a straightforward diazotization and coupling method[12].

Sulfonamide-based compound, of the chemical formula $SO_2NH_2$, was the first medicine to be extensively utilized on a large scale as a disease prevention and chemotherapeutic agent[13]. There are two sorts of sulfa-pharmaceuticals, antibiotics and non-antibiotic sulfa drugs. During the clinical examination of sulfonamides, several non-antibiotic sulfonamides were found to be useful for managing a range of illnesses involving diabetes[14]. Sulfamethoxazole has been connected to oral hypoglycemic sulfonylurea, a scaffold that stimulates insulin release. Also, sulfamethoxazole and its derivatives may be a promising lead compound for developing new anti-diabetic medicines[15]. As a result, scientists are increasingly focused on the creation of new sulfamethoxazole- and sulfonamide-based derivatives with antioxidant and hypoglycemic characteristics for medical and pharmaceutical applications[16].

Our goal is to develop novel azo-sulfamethoxazole derivatives and evaluate their antioxidant characteristics by assessing their capacity to trap DPPH- and hydroxyl-free radicals while also contributing an electron in an oxidation-reduction process. In addition, the hypoglycemic effect against yeast-glucosidase and porcine-amylase was assessed *in vitro*.

## Experimental

### General aspects

The reagents, chemicals, and solvents needed to perform the synthesis of **SMZ** derivatives and evaluate their antioxidant and hypoglycemic properties were bought from a variety of worldwide sources, including Chem-Lab, Scharlau, Labcorp, Sigma-Aldrich, and Haihang. On the basis of the open-capillary approach, the melting points (mp) of the manufactured **SMZ** derivatives were reported using the electrically heated IA9300 electronic melting point device. The approach named "Thin-layer chromatography" (TLC) was used to follow the implementation of the synthetic reactions and to show the purity of the generated **SMZ** derivatives. In this approach, the mobile and stationary portions were Millipore SigmaTM TLC-Silica (F-254) Gel 60 and $CH_3Cl$:MeOH (5:1) combination, correspondingly. Standard spectrophotometers namely the Bruker Avance-3 HD (300 MHz for proton and 75 MHz for carbon) using deuterated DMSO as a solvent, Bruker FTIR-α-ATR, and Bio-Cary 300 UV-Vis were used to determine the $^1$H-NMR, $^{13}$C-NMR, IR, and $\lambda_{max}$ ranges of the generated **SMZ** derivatives.

### Synthesis of the target derivatives SMZ 1-8

In a beaker, 1.265 g of **SMZ** (5 mmol) was dissolved in a combination of equal volume (10 ml) of conc. HCl and $H_2O$. The obtained solution was chilled by dipping in a crushed ice bath, and the reaction temperature was maintained under 5°C across the reaction. The chilled aqueous solution of natrium nitrit (5 ml, 8.28 %) was poured into a dropping funnel and dropped into the agitated acidic solution of **SMZ** in an ice bath. The reaction temperature was maintained below 10°C by introducing several grams of cracked ice as required[17].

The resultant solution was agitated for 5 min in an ice bath after the last addition. A combination consisting of a drop of this solution and 4 drops of $H_2O$ was applied on a KI-starch paper. If no immediately blue color was generated at the application site, 1 ml of natrium nitrit aqueous solution (8.28%) was added, and the solution was examined after 5 min. More addition and monitoring were carried out till an instantaneous blue coloration was attained to afford the diazonium salt solution of **SMZ**[18].

In a beaker submerged in an ice bath, the phenol-based compound (5 mmol) was treated with 10 ml of 10% aqueous NaOH to obtain the phenolic solution. This solution was agitated and the reaction temperature was maintained below

5°C with the help of the direct addition of broken ice. The chilled diazonium salt solution was poured inside a dropping funnel with several grams of broken ice and dropped to the agitated phenolic solution in an ice bath. The colored solid particles were segregated, filtered, washed with cold $H_2O$ several times, and purified by re-crystallizing from EtOH[19].

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(4-Hydroxyphenyl) diazenyl)-benzenesulfonamide (SMZ-1)**: mp= 180-182°C; $\lambda_{max}$ (EtOH)= 436 nm; %yield= 52; $R_f$= 0.52; FTIR (v, stretching, $cm^{-1}$): 3361 (N-H, sulfonamide), 3210 (O-H, phenolic), 3046 (C-H, alkene), 2894 (C-H, alkane), 1656 (C=C, isoxazole), 1622 (C=N, isoxazole), 1558 (C=C, aromatic), and 1428 (N=N, azo); $^1$H-NMR: δ= 9.02 (2H, d, H-9 and H-9', *J*=6 Hz), 8.24 (2H, d, H-8 and H-8', *J*=6 Hz), 7.97 (2H, d, H-13 and H-17, *J*=6 Hz), 7.18 (2H, d, H-14 and H-16, *J*=6 Hz), 6.11 (1H, s, H-4), 5.26 (1H, s, OH-15), 4.12 (1H, s, H-6), and 2.42 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.3 (C, C-5), 161.8 (C, C-15), 154.9 (C, C-10), 151.2 (C, C-3), 146.6 (C, C-12), 143.1 (C, C-7), 129.4 (CH, C-8 and C-8'), 126.7 (CH, C-13 and C-17), 124.9 (CH, C-9 and C-9'), 119.4 (CH, C-14 and C-16), 98.0 (CH, C-4), and 14.6 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3yl)-(2,4-Dihydroxyphenyl) diazenyl)-benzenesulfonamide (SMZ-2)**: mp= 202-204°C; $\lambda_{max}$ (EtOH)= 487 nm; %yield= 86; $R_f$= 0.38; FTIR (v, stretching, $cm^{-1}$): 3360 (N-H, sulfonamide), 3213 (O-H, phenolic), 3042 (C-H, alkene), 2890 (C-H, alkane), 1655 (C=C, isoxazole), 1623 (C=N, isoxazole), 1556 (C=C, aromatic), and 1425 (N=N, azo); $^1$H-NMR: δ= 9.01 (2H, d, H-9 and H-9', *J*=6 Hz), 8.25 (2H, d, H-8 and H-8', *J*=6 Hz), 7.80 (1H, d, H-17, *J*=6 Hz), 6.86 (1H, s, H-14), 6.74 (1H, d, H-16, *J*=6 Hz), 6.12 (1H, s, H-4), 5.26 (1H, s, OH-15), 5.12 (1H, s, OH-13), 4.13 (1H, s, H-6), and 2.42 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.3 (C, C-5), 163.2 (C, C-15), 155.9 (C, C-13), 154.6 (C, C-10), 151.2 (C, C-3), 143.2 (C, C-7), 129.4 (CH, C-8 and C-8'), 128.1 (C, C-17), 124.9 (CH, C-9 and C-9'), 119.0 (C, C-12), 112.0 (CH, C-16), 107.1 (CH, C-14), 98.0 (CH, C-4), and 14.8 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(4-Hydroxy-2-methoxyphenyl)diazenyl)-benzenesulfonamide (SMZ-3)**: mp= 190-192°C; $\lambda_{max}$ (EtOH)= 480 nm; %yield= 82; $R_f$= 0.56; FTIR (v, stretching, $cm^{-1}$): 3356 (N-H, sulfonamide), 3210 (O-H, phenolic), 3040 (C-H, alkene), 2915, 2890 (C-H, alkane), 1658 (C=C, isoxazole), 1621 (C=N, isoxazole), 1555 (C=C, aromatic), 1426 (N=N, azo), and 1216, 1057 (C-O-C, aryl-alkyl ether); $^1$H-NMR: δ= 9.03 (2H, d, H-9 and H-9', *J*=6 Hz), 8.26 (2H, d, H-8 and H-8', *J*=6 Hz), 7.86 (1H, d, H-17, *J*=6 Hz), 6.90 (1H, s, H-14), 6.73 (1H, d, H-16, *J*=6 Hz), 6.12 (1H, s, H-4), 5.25 (1H, s, OH-15), 4.14 (1H, s, H-6), 3.94 (3H, s, $OCH_3$-13), and 2.44 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.4 (C, C-5), 162.8 (C, C-15), 156.9 (C, C-13), 154.5 (C, C-10), 151.1 (C, C-3), 143.3 (C, C-7), 129.2 (CH, C-8 and C-8'), 127.7 (C, C-17), 124.6 (CH, C-9 and C-9'), 111.7 (CH, C-16), 117.4 (C, C-12), 105.5 (CH, C-14), 98.2 (CH, C-4), 56.6 ($CH_3$, $OCH_3$-13), and 14.5 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(4-Hydroxy-2-methylphenyl)diazenyl)-benzenesulfonamide (SMZ-4)**: mp= 209-211°C; $\lambda_{max}$ (EtOH)= 425 nm; %yield= 76; $R_f$= 0.53; FTIR (v, stretching, $cm^{-1}$): 3353 (N-H, sulfonamide), 3212 (O-H, phenolic), 3036 (C-H, alkene), 2895 (C-H, alkane), 1657 (C=C, isoxazole), 1622 (C=N, isoxazole), 1558 (C=C, aromatic), and 1427 (N=N, azo); $^1$H-NMR: δ= 9.02 (2H, d, H-9 and H-9', *J*=6 Hz), 8.28 (2H, d, H-8 and H-8', *J*=6 Hz), 7.85 (1H, d, H-17, *J*=6 Hz), 7.33 (1H, s, H-14), 6.98 (1H, d, H-16, *J*=6 Hz), 6.14 (1H, s, H-4), 5.26 (1H, s, OH-15), 4.13 (1H, s, H-6), 2.73 (3H, s, $CH_3$-13), and 2.44 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.2 (C, C-5), 160.2 (C, C-15), 154.6 (C, C-10), 151.4 (C, C-3), 145.9 (C, C-12), 143.3 (C, C-7), 141.8 (C, C-13), 129.2 (CH, C-8 and C-8'), 124.6 (CH, C-9 and C-9'), 120.3 (CH, C-16), 119.4 (C, C-17), 118.2 (CH, C-14), 98.2 (CH, C-4), 20.1 ($CH_3$, $CH_3$-13), and 14.6 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(2-Fluoro-4-hydroxyphenyl)diazenyl)-benzenesulfonamide (SMZ-5)**: mp= 220-222°C; $\lambda_{max}$ (EtOH)= 466 nm; %yield= 42; $R_f$= 0.40; FTIR (v, stretching, $cm^{-1}$): 3357 (N-H, sulfonamide), 3211 (O-H, phenolic), 3046 (C-H, alkene), 2884 (C-H, alkane), 1655 (C=C, isoxazole), 1621 (C=N, isoxazole), 1557 (C=C, aromatic), 1426 (N=N, azo), and 1092 (C-F); $^1$H-NMR: δ= 9.04 (2H, d, H-9 and H-9', *J*=6 Hz), 8.30 (2H, d, H-8 and H-8', *J*=6 Hz), 7.95 (1H, d, H-17, *J*=6 Hz), 7.46 (1H, s, H-14), 6.94 (1H, d, H-16, *J*=6 Hz), 6.16 (1H, s, H-4), 5.29 (1H, s, OH-15), 4.13 (1H, s, H-6), and 2.45 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.0 (C, C-5), 163.4 (C, C-15), 158.3 (C, C-13), 154.7 (C, C-10), 151.5 (C, C-3), 143.5 (C, C-7), 134.4 (C, C-12), 129.1 (CH, C-8 and C-8'), 128.3 (C, C-17), 124.8 (CH, C-9 and C-9'), 115.1 (CH, C-16), 107.5 (CH, C-14), 98.2 (CH, C-4), and 14.6 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(2-Chloro-4-hydroxyphenyl)diazenyl)-benzenesulfonamide (SMZ-6)**: mp= 196-198°C; $\lambda_{max}$ (EtOH)= 441 nm; %yield= 47; $R_f$= 0.42; FTIR (v, stretching, $cm^{-1}$): 3354 (N-H, sulfonamide), 3216 (O-H, phenolic), 3046 (C-H, alkene), 2886 (C-H, alkane), 1654 (C=C, isoxazole), 1623 (C=N, isoxazole), 1553 (C=C, aromatic), 1427 (N=N, azo), and 984 (C-Cl); $^1$H-NMR: δ= 9.05 (2H, d, H-9 and H-9', *J*=6 Hz), 8.28 (2H, d, H-8 and H-8', *J*=6 Hz), 7.91 (1H, d, H-17, *J*=6 Hz), 7.51 (1H, s, H-14), 7.05 (1H, d, H-16, *J*=6 Hz), 6.15 (1H, s, H-4), 5.29 (1H, s, OH-15), 4.13 (1H, s, H-6), and 2.44 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.2 (C, C-5), 159.7 (C, C-15), 154.6 (C, C-10), 151.4 (C, C-3), 147.8 (C, C-12), 143.5 (C, C-7), 132.2 (C, C-13), 129.1 (CH, C-8 and C-8'), 128.1 (C, C-17), 124.7 (CH, C-9 and C-9'), 120.2 (CH, C-14), 117.6 (CH, C-16), 98.3 (CH, C-4), and 14.6 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(2-Bromo-4-hydroxyphenyl)diazenyl)-benzenesulfonamide (SMZ-7)**: mp= 187-189°C; $\lambda_{max}$ (EtOH)= 429 nm; %yield= 48; $R_f$= 0.44;

FTIR (v, stretching, $cm^{-1}$): 3353 (N-H, sulfonamide), 3210 (O-H, phenolic), 3043 (C-H, alkene), 2885 (C-H, alkane), 1655 (C=C, isoxazole), 1622 (C=N, isoxazole), 1556 (C=C, aromatic), 1426 (N=N, azo), and 901 (C-Br); $^{1}$H-NMR: δ= 9.04 (2H, d, H-9 and H-9', *J*=6 Hz), 8.27 (2H, d, H-8 and H-8', *J*=6 Hz), 7.86 (1H, d, H-17, *J*=6 Hz), 7.56 (1H, s, H-14), 7.11 (1H, d, H-16, *J*=6 Hz), 6.16 (1H, s, H-4), 5.31 (1H, s, OH-15), 4.13 (1H, s, H-6), and 2.44 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.3 (C, C-5), 159.6 (C, C-15), 154.5 (C, C-10), 151.5 (C, C-3), 150.7 (C, C-12), 143.5 (C, C-7), 129.1 (CH, C-8 and C-8'), 128.9 (C, C-17), 124.6 (CH, C-9 and C-9'), 122.7 (CH, C-14), 118.5 (CH, C-16), 115.3 (C, C-13), 98.3 (CH, C-4), and 14.5 ($CH_3$, C-5') ppm.

**(*E*)-4-(N-(5-methylisoxazol-3-yl)-(2-Iodo-4-hydroxyphenyl)diazenyl)-benzenesulfonamide (SMZ-8)**: mp= 177-179°C; $\lambda_{max}$ (EtOH)= 428 nm; %yield= 49; $R_f$= 0.45; FTIR (v, stretching, $cm^{-1}$): 3355 (N-H, sulfonamide), 3212 (O-H, phenolic), 3044 (C-H, alkene), 2883 (C-H, alkane), 1654 (C=C, isoxazole), 1625 (C=N, isoxazole), 1559 (C=C, aromatic), 1428 (N=N, azo), and 826 (C-I); $^{1}$H-NMR: δ= 9.04 (2H, d, H-9 and H-9', *J*=6 Hz), 8.27 (2H, d, H-8 and H-8', *J*=6 Hz), 7.77 (1H, s, H-14), 7.68 (1H, d, H-17, *J*=6 Hz), 7.16 (1H, d, H-16, *J*=6 Hz), 6.15 (1H, s, H-4), 5.30 (1H, s, OH-15), 4.12 (1H, s, H-6), and 2.45 (3H, s, H-5') ppm; $^{13}$C-NMR: δ= 170.4 (C, C-5), 163.4 (C, C-15), 156.3 (C, C-12), 154.5 (C, C-10), 151.5 (C, C-3), 143.6 (C, C-7), 129.2 (CH, C-8 and C-8'), 128.3 (C, C-17), 125.9 (CH, C-14), 124.7 (CH, C-9 and C-9'), 118.7 (CH, C-16), 98.4 (CH, C-4), 87.3 (C, C-13), and 14.5 ($CH_3$, C-5') ppm.

### Assessment of the antioxidant activity

The amplitudes of the generated **SMZ** derivatives to liquidate the free radicals of DPPH (1,1-diphenyl-2-picryl-hydrazyl) and hydroxyl moieties, and donate an electron in redox reaction were monitored utilizing L-ascorbic acid (**Vit. C**) as a standard. From a parent mixture (2 mg/ml), solutions of the following ten concentrations were prepared using DMSO as a thinner: 1000, 750, 500, 250, 125, 100, 50, 25, 12.5, and 6.25 µg/ml. For each tested entity including **SMZ**, standard, and generated **SMZ** derivatives, the liquidating percentage (L%) values of the applied concentrations were quantified based on the following mathematical equation:

$$L(\%) = Abs_{control} - Abs_{sample} \div Abs_{control} \times 100$$

The signifiers $Abs_{control}$ and $Abs_{sample}$ referred to the observed absorptions at a defined colored wavelength of the control and tested sample, respectively [20].

The measure named liquidating activity was symbolized as $LC_{50}$ and represented the concentration of the chemical entity at which half of the free radicals was neutralized or half of the oxidized iron particles was reduced. This measure was specified by plotting the linkage among **L%** values and their corresponding logarithmic concentrations through a non-linear regression [21].

#### *DPPH-free radical liquidating assay*

The examined solution (1.5 ml) was combined at a specific concentration with ethanolic DPPH solution (0.5 ml, 0.1 mM). To shield it from daylight, the combined solution was layered by aluminum platelet, and the covered system was held at 25°C for half an hour. The capacity of the examined solution to obliterate the DPPH violet color was assessed colorimetrically at 517 nm. The control solution was composed of 0.5 ml ethanolic DPPH solution plus 1.5 ml of the diluting solvent[22].

#### *Hydroxyl-free radical liquidating assay*

The examined solution (1.5 ml) at a defined concentration was combined with 2.4 ml of the buffer named potassium phosphate (0.2M, pH 7.8). To this combination, 60 µl of 0.001 M ferric chloride, 90 µl of 0.001 M pyridino[3,2-h] quinoline, and 150 µl of 0.17 M hydrogen dioxide were added successively. The resulted mixture was maintained at 25°C for 5 min and examined colorimetrically at 560 nm. The control solution was composed of all the above-mentioned components, but the examined solution was replaced with the diluting solvent[23].

#### *Total reducing capacity*

The examined solution (1 ml) at a defined concentration was combined with 2 ml of the buffer named potassium phosphate (0.2M, pH 6.6) and 2 ml aqueous potassium ferricyanide solution (1%). The combination was kept for 20 min in a thermo-digital water bath monitored at 50°C. Afterthought, the progress of the interaction was arrested by adding 2 ml aqueous trichloroacetic acid solution (10%). The resultant mixture was centrifuged at 2000 rpm for 10 min. To a combination of 2 ml $H_2O$ and 0.4 ml aqueous ferric chloride solution (0.01%), a 2 ml supernatant was added. The resulted combination was kept at 25°C for 10 min and assessed colorimetrically at 700 nm. The control solution was prepared as the tested mixture but the examined solution was replaced with the diluting solvent[24].

### Assessment of the hypoglycemic activity

This effect was assessed *in vitro* by examining the inhibitory impact of the generated **SMZ** derivatives versus two enzyme phenotypes known to be involved in controlling the blood glucose level, namely yeast α-glucosidase and porcine α-amylase. The measure used to specify this effect termed $IC_{50}$, which is defined as the concentration of the generated **SMZ** derivative required to inhibit the enzyme activity by 50% under test conditions. Before initiating these two assays, various concentrations were prepared from a parent solution (2 mg/ml). The prepared concentrations including 1000, 500, 250, 125, 100, 50, 25, 12.5, and 6.25 µg/ml were prepared by using DMSO as a thinner[25].

### *Yeast α-glucosidase inhibiting assay*

An equal volume (20 µl) of specific concentration of the examined solution and phosphate buffer solution containing α-glucosidase enzyme (0.1 unit/ml) were mixed. The substrate compound named 4-nitrophenyl-α-D-glucopyranoside was dissolved in a phosphate buffer solution (pH 6.8) achieving a final concentration of 375 µM. Subsequently, 40 µl of the substrate solution was added and incubated with the prepared test solution-enzyme mixture at 37°C for half an hour. The progress of the interaction was arrested by adding phosphate buffer solution containing $Na_2CO_3$ (80 µl, 0.2 M) to the tested mixture. The capacity of the **SMZ** derivative to inhibit the enzyme activity was detected colorimetrically at 405 nm, and the percentage of this inhibition was calculated by applying the incoming formula:

$$\alpha - Glucosidase\ inhibitory\ \% = Abs_{control} - Abs_{sample} \div Abs_{control} \times 100$$

Acarbose was employed as a standard, while the control solution was prepared as the tested mixture but the examined solution was replaced with DMSO [26].

### *Porcine α-amylase inhibiting assay*

An equal volume (20 µl) of specific concentration of the examined solution and phosphate buffer solution containing α-amylase enzyme (2 unit/ml) were mixed. The substrate compound named starch was dissolved in a phosphate buffer solution (pH 6.8) achieving a final volume of 2 ml with a concentration of 0.5 mM. Subsequently, the tested mixture was incubated at 25°C for 10 min. The progress of the interaction was arrested by adding 2 ml of finishing reagent, which was a 0.4 M aqueous NaOH solution containing 3,5-dinitrosalicylic acid (1%) and anhydrous sodium potassium tartrate (12%). The resultant mixture was heated in a boiling water bath for 15 min, diluted with $H_2O$ achieving the final volume of 10 ml, and cooled to 25°C using an aqueous ice bath. The capacity of the examined solution to inhibit the enzyme activity was measured colorimetrically at 540 nm, and the percentage of this inhibition was calculated by applying the incoming formula:

$$\alpha - Amylase\ inhibitory\ \% = Abs_{control} - Abs_{sample} \div Abs_{control} \times 100$$

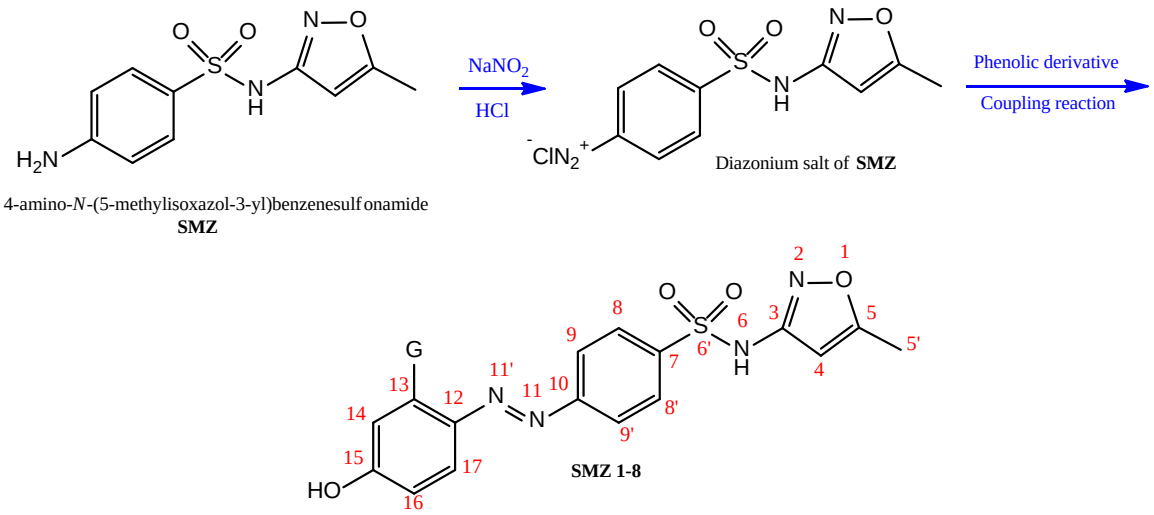
Acarbose was employed as a standard, while the control solution was prepared as the tested mixture but the examined solution was replaced with DMSO[27].

## Results and Discussion

### Chemical synthesis

In a synthesis of **SMZ** derivatives, natrium nitrit was used to transform **SMZ** to its corresponding diazonium salt. The latter was then coupled with various phenolic-based compounds to afford the target **SMZ** derivatives [28,29]. One of the most observable characteristics in the synthesis was the significant variation in the %yield. The order of decreasing regarding this percentage was **SMZ-2**, **-3**, **-4**, **-1**, **-8**, **-7**, **-6**, and **-5**. The authors attributed this feature to the nucleophilicity of the phenolic aromatic ring that decreases in the same direction [30–33]. Scheme 1 illustrates the schematic preparative steps utilized in the synthesis of the target **SMZ** derivatives.

**Scheme 1. Schematic representation for synthesizing SMZ derivatives.**




**SMZ-1**: G = H, **SMZ-2**: G = OH, **SMZ-3**: G = $OCH_3$, **SMZ-4**: G = $CH_3$,
**SMZ-5**: G = F, **SMZ-6**: G = Cl, **SMZ-7**: G = Br, **SMZ-8**: G = 1

### Assessment of the antioxidant activity

Antioxidant research has gotten a lot of attention in the medical current history because of its possible involvement in the prevention and control of numerous diseases that influence human health [34,35]. Since the link connecting oxidative stress and diabetes is undeniable, the development of new synthetic antioxidants has attracted the medical community's interest[36–38].

The antioxidant potential of the target **SMZ** derivatives was determined by observing their ability to capture DPPH and hydroxyl reactive species and contribute an electron in the electron transfer oxidation-reduction process.

The results, presented in Table 1 and illustrated graphically in Figure 1, showed four noticeable aspects. The first is that **SMZ** had low antioxidant activity under the circumstances of the tests employed. Second, replacing the primary aromatic amine of **SMZ** with the phenolic azo moiety resulted in a considerable elevation in the **SMZ**'s antioxidant activity[39]. Third, the synthesized **SMZ** derivatives followed a similar pattern in their ability to quench the utilized reactive species and donate an electron in the applied redox reaction. This pattern is **SMZ-2**, **SMZ-6**, **SMZ-3**, **SMZ-4**, **SMZ-1**, **SMZ-8**, **SMZ-7**, and **SMZ-5**. From this order, the authors concluded that the electron releasing/withdrawing property of the group ortho to the aromatic azo moiety has a significant influence on the antioxidant activity of the synthesized **SMZ** derivatives. Accordingly, except for **SMZ-6**, the presence of the electron-donating group in that position has a positive impact, while the electron-withdrawing group plays a negative role[40]. Also, the antioxidant ac xcvbnminol-related ring in the chemical backbone of this comp≥,mnbvc zxcvb cv mnbvc zxcound. The relation between this structural feature and the antioxidant activity has been also documented in many isolated natural products[31].

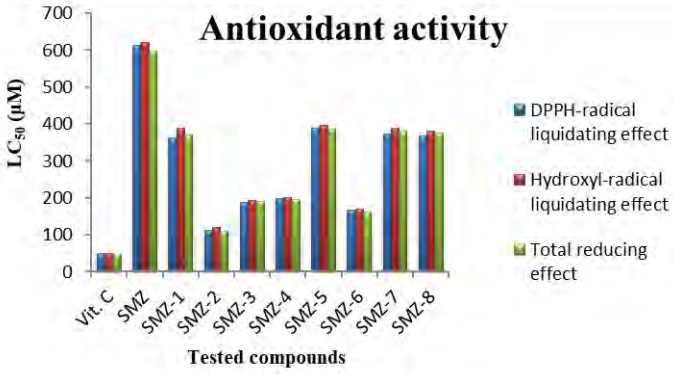


**Table 1. The findings of three independent experiments used to investigate the antioxidant capacity of the SMZ and its synthesized derivatives.**

| Symbol | $LC_{50}$±SD | | |
|---|---|---|---|
| | DPPH-radical liquidating effect | Hydroxyl-radical liquidating effect | Total reducing effect |
| **Vit. C** | 48.59 ± 0.89 | 50.35 ± 0.82 | 48.19 ± 0.94 |
| **SMZ** | 612.46 ± 0.81 | 620.38 ± 0.88 | 597.92 ± 1.04 |
| **SMZ-1** | 361.70 ± 0.82 | 387.61 ± 0.93 | 369.14 ± 1.02 |
| **SMZ-2** | 112.58 ± 0.94 | 118.92 ± 0.91 | 109.55 ± 1.07 |
| **SMZ-3** | 187,12 ± 0.96 | 192.73 ± 0.89 | 190.37 ± 0.86 |
| **SMZ-4** | 197.91 ± 0.79 | 201.04 ± 0.91 | 194.83 ± 0.94 |
| **SMZ-5** | 389.18 ± 0.85 | 396.03 ± 0.84 | 386.25 ± 1.02 |
| **SMZ-6** | 166.48 ± 1.02 | 170.16 ± 0.92 | 161.44 ± 1.08 |
| **SMZ-7** | 373.17 ± 1.05 | 388.68 ± 0.98 | 380.56 ± 0.89 |
| **SMZ-8** | 366.59 ± 0.81 | 380.34 ± 0.98 | 376.12 ± 0.90 |

*$LC_{50}$ was quantified in µg/ml, and each run was accomplished in three separate trials (n=3).*

**Figure 1. Diagrammatic representation of the findings acquired from testing the antioxidant activity of the SMZ and its synthesized derivatives.**




### Assessment of the hypoglycemic activity

Diabetes mellitus (**DM)** is one of the most riskiest-, exhausted-, chronic-diseases that is developing worldwide with a substantial rise in obesity, unhealthy lifestyle, and aging[42]. One approach for dealing with this metabolic disorder is through interfering with its pathogenesis involving the aberrant releasing of reactive harmful molecules[43]. Based on this approach and after assessing their antioxidant activity, the hypoglycemic impact of the synthesized **SMZ** derivatives was explored. This impact was detected by monitoring the strength of these derivatives to suppress two enzymes involved in the regulation of blood glucose, namely porcine α-amylase and yeast α-glucosidase.

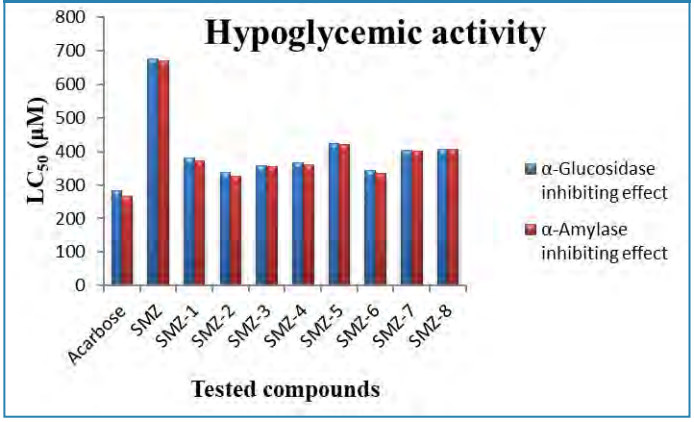
The findings, which are provided in Table 2 and visually shown in Figure 2, revealed four remarkable points. First, considering the conditions of the conducted experiments and comparing with standard, **SMZ** exhibited a weak hypoglycemic impact, but its synthetic derivatives had a moderate-to-good activity. Second, substituting the phenolic azo moiety for the primary aromatic amine of **SMZ** caused a powerful improvement in its hypoglycemic efficacy. Third, the order, which is closely related to that reported for the antioxidant activity, of hypoglycemic effect concerning the synthesized **SMZ** derivatives followed a comparable modality in their capacity for inhibiting the two test enzymes[44]. Four, of the synthesized **SMZ** derivatives, the **SMZ-2** has the greatest hypoglycemic impact, which is roughly comparable to that of the standard.

**Table 2. The results of three different runs utilized to study the hypoglycemic activity of SMZ and its synthetic derivatives.**

| Symbol | $IC_{50}$±SD | |
|---|---|---|
| | α-Glucosidase inhibiting effect | α-Amylase inhibiting effect |
| **Acarbose** | 284.16 ± 0.93 | 265.34 ± 0.90 |
| **SMZ** | 675.21 ± 1.04 | 669.04 ± 0.96 |
| **SMZ-1** | 380.34 ± 1.01 | 372.27 ± 1.05 |
| **SMZ-2** | 337.46 ± 0.96 | 326.02 ± 1.03 |
| **SMZ-3** | 356.38 ± 0.99 | 354.19 ± 1.03 |
| **SMZ-4** | 365.79 ± 0.98 | 360.93 ± 0.89 |
| **SMZ-5** | 422.62 ± 0.97 | 419.49 ± 0.90 |
| **SMZ-6** | 342.68 ± 1.05 | 335.16 ± 1.01 |
| **SMZ-7** | 402.70 ± 1.03 | 401.11 ± 0.98 |
| **SMZ-8** | 407.54 ± 1.04 | 405.08 ± 0.91 |

*$IC_{50}$ was quantified in µg/ml, and each trials was accomplished in three separate trials (n=3).*

**Figure 2. Diagrammatic representation of the outcomes obtained from assessing the hypoglycemic activity of the SMZ and its synthesized derivatives.**




## Conclusion

The use of **SMZ** as a starting material to produce eight-based derivatives was successful in this study. The observations indicated that the synthesized **SMZ** derivatives, particularly **SMZ-2** and **SMZ-6**, had substantial antioxidant and hypoglycemic characteristics when matched to **SMZ** and standards, suggesting them potential candidates for treating diabetes. These derivatives also offered a new functional scaffold that could be used to create new bioactive medicines to address this metabolic ailment.

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